

PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

MICHAEL J. ZIGMOND
ROBERT E. BURKE

Parkinson's disease (PD) is thought to affect more than 1 million people in the United States alone, 1 of every 100 individuals above the age of 55. In the two centuries since it was first described by James Parkinson, we have learned a great deal about the disorder. We have, for example, learned where the primary lesion is and what many of the clinical manifestations are. However, it has only been in the past few decades that insights have begun to emerge regarding the cause of the disease, and only now can one begin to see the possibilities of treatments emerging that will provide more than temporary symptomatic relief. Beginning with the Nobel Prize-winning work of Arvid Carlsson, which pointed to the loss of dopamine (DA) as the principal deficit in PD and to levodopa as a mode of pharmacotherapy, we have come to understand what fails in this disorder and, more recently, how we might correct that failure. Moreover, although Parkinson focused entirely on motor symptoms, we have come to realize that the disorder is much more complex and includes a panoply of psychiatric symptoms as well.

The clinical features, course, and treatment of PD are presented in detail in Chapters 122 and 124; thus, for the purposes of this review of etiology and pathogenesis, we only briefly highlight the more important aspects of these topics, including those of a psychiatric nature. We then describe the pathology before turning to several promising leads with regard to the underlying etiology of the disorder.

CLINICAL SIGNS AND SYMPTOMS

Motor Manifestations

PD is a chronic, progressive neurologic disease. It presents with four cardinal motor manifestations: tremor at rest, ri-

gidity, bradykinesia (or slowing of movement), and postural instability. Not all patients initially present with all of the classic signs of the disorder; there may be only one or two. Often, the first complaint is one of motor weakness or stiffness, and the cause is commonly misdiagnosed. However, postural deficits and tremor may soon emerge, prompting a reconsideration of the basis of the problem. It is important to note, however, that the clinical diagnosis of PD is made on the basis of a medical history and neurologic examination; there is currently no laboratory test that can definitely establish a diagnosis. Even neuroimaging, which can be used to obtain an estimate of DA loss (15,128), is imperfect and in any event is too expensive to be used as a routine diagnostic tool. As a result, it has been estimated that a significant number of individuals diagnosed as having PD fail to show the histopathologic hallmarks of the disease upon autopsy (48,70,134).

A *tremor* at rest is one of the most characteristic features of the disease, occurring in 70% of patients (68). Whereas it is not required for diagnosis, the prolonged absence of tremor in the course of a patient's illness should lead to the careful consideration of other neurologic conditions that can present with signs of parkinsonism, including the multiple system atrophies, progressive supranuclear palsy, corticobasal ganglionic degeneration, and others (94). *Rigidity* is a motor sign more often appreciated by the examining physician than the patient; it is detected as a resistance to passive movement of the limbs. It is often uniform in directions of flexion and extension ("lead pipe rigidity"), but there may be a superimposed ratcheting ("cogwheel rigidity"). *Bradykinesia* refers to a slowness and paucity of movement; examples include loss of facial expression, which may be misinterpreted as a loss of affect, and associated movements such as arm swinging when walking. Bradykinesia is not due to limb rigidity; it can be observed in the absence of rigidity during treatment. When bradykinesia affects the oropharynx, it can lead to difficulties in swallowing, which in turn may cause aspiration pneumonia, a potentially life-threatening complication. Of the cardinal motor signs, *pos-*

Michael J. Zigmond: Departments of Neurology and Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania.

Robert E. Burke: Department of Neurology, Columbia University, New York, New York.

tural instability is the most potentially dangerous, because it can lead to falls with resulting fractures. It is also one of the manifestations that responds less well to levodopa therapy. Tremor may also be less responsive, even early in the course (95).

An additional motor feature of PD is the freezing phenomenon, also referred to as “motor block” (51). In its most typical form, freezing occurs as a sudden inability to step forward while walking. It may occur at the beginning (“start hesitation”), at a turn, or just before reaching the destination. It is transient, lasting seconds or minutes, and suddenly abates. Combined with postural instability, it can be devastating. Freezing does not always improve with levodopa, and, in fact, can be made worse.

Cognitive and Psychiatric Manifestations

It is increasingly clear that there are many parallel circuits within the basal ganglia, each subserving a different function and each modulated by DA (see Chapter 122). Thus, it is reasonable to predict that patients will have a wide variety of dysfunctions extending well beyond the classic motor disabilities associated with the disease. Indeed, patients with Parkinson’s disease appear to be at increased risk for a variety of cognitive and psychiatric dysfunctions. Most common is dementia and depression. However, hallucinations, delusions, irritability, apathy, and anxiety also have been reported (1). Here we will comment on the most prevalent of these symptoms.

Dementia is now recognized as one of the cardinal non-motor manifestations of PD. It is a major cause of disability, and, unlike the motor manifestations, there currently is no effective symptomatic treatment. Aarsland and co-workers (2) identified in dementia in 28% of PD patients. The prevalence depends on age; in a study of PD patients over the age of 85 by Mayeux et al. (108), 65% were demented. PD patients with dementia show a more rapidly progressive course (110), and are more likely to be institutionalized, than nondemented individuals (2).

Years ago, there was debate about whether *depression* is a primary manifestation of PD or a reaction to having a chronic neurologic illness. There is now little question that it is a primary manifestation. Mayeux and colleagues (109a) have found that 47% of PD patients show evidence of depression, and some have found an even higher incidence (147). Moreover, Aarsland and colleagues (2) report that major depression is much more common among PD patients who also have signs of dementia (22%) than those who did not (2%). The depression, however, is not related to the severity of motor signs; indeed, many patients are depressed prior to the onset of frank neurologic dysfunction. Moreover, the depression is often greater than that seen in individuals with comparably debilitating motor dysfunction due to other disorders.

It has long been suggested that patients with PD can

have particular premorbid *personality traits* (126,129,152). For example, some have argued that they tend to follow socially approved paths, are more introverted, and have less addictive personalities (e.g., are less inclined to smoke or drink) (158). Support for this hypothesis comes from a number of studies, including several involving twins that are discordant for Parkinson’s disease (65,128). Many of these studies suffer from such problems as small sample size and retrospective analysis. Nonetheless, as noted at the outset of this section, the anatomy of basal ganglia circuitry is consistent with a broad range of functions, and some of these could easily affect personality in subtle ways. Might one, posit, for example, a “rigid PD personality” that parallels the rigid PD motor capacity? The issue is an important one, not only for our understanding of PD and of the neurobiology of behavior, but also because of the value of developing diagnostic screens that will permit the detection of PD at the earliest possible stage. Such early detection will become increasingly important as neuroprotective strategies emerge.

Other Manifestations

In addition to these neurologic signs and symptoms, PD patients often have disturbing sensory symptoms and pain in affected limbs. Many PD patients also have signs of autonomic failure, including orthostatic hypertension, constipation, urinary hesitancy, and impotence in men (90,107,133).

PATHOLOGY

Neuron Loss

A hallmark pathologic feature of PD, and essential for its pathologic diagnosis, is loss of DA neurons of the substantia nigra pars compacta (SNpc). At the time of death, even mildly affected PD patients have lost about 60% of their DA neurons, and it is this loss, in addition to possible dysfunction of the remaining neurons, that accounts for the approximately 80% loss of DA in the corpus striatum. We have discussed the basis for the need for extensive loss of DA neurons before the emergence of gross neurologic deficits in the previous edition of this series (5) and elsewhere (164,165). Briefly, our conception is as follows: As the terminals of DA neurons degenerate, there is a reduction in high-affinity DA uptake. This, coupled with some inherent redundancy in DA terminals and DA receptors, appears to permit striatal function to continue without disruption or active compensation during early phases of the neurodegenerative process. After somewhat larger lesions, the remaining DA terminals appear to increase the amount of transmitter synthesized and delivered to the extracellular fluid. This seems to be due at least in part to a net increase in the amount of DA released in response to terminal depolarization, a consequence of the transient disruption of the homeostatic regulatory systems that exist within the affected

systems (164). Once released, a portion of the DA appears to diffuse out of the synapse and into the extracellular space, where its actions are prolonged due to the relative absence of high-affinity DA uptake sites. We hypothesize that these events permit the SNpc to continue to exert dopaminergic control over striatal cell function as long as some minimal number of DA terminals remain. However, the increased synthesis and release of DA may increase reactive metabolites formed from DA and thus contribute to the progression of the disease (see below).

Neurologic deficits emerge when the availability of DA falls below the level required for rapid compensation or when the system is subjected to certain pharmacologic, environmental, or physiologic challenges. These can subside if additional, slowly developing compensations, such as the synthesis and insertion of additional DA receptors, the induction of tyrosine hydroxylase (TH) synthesis, sprouting, or regeneration, occur at a more rapid rate than does the underlying neurodegeneration. This has been observed to be the case in animal models, wherein recovery often occurs after the abrupt loss of even 90% of striatal DA, as occurs in most animal models. In patients, however, where the degenerative process is typically progressive, such recovery would not be expected to occur spontaneously. However, an important implication of the ability of brain to compensate for partial loss of DA neurons in these ways is that once deficits do occur, the task of medicine need not be to restore the entire nigrostriatal projection but only the far less daunting objective of returning the availability of DA to the level required to attain the preclinical state.

Although there are several groups of dopaminergic neurons in the central nervous system (CNS), it is the loss of DA cells in the SNpc that is believed to account for all of the motor manifestations of PD. This clinicopathologic correlation is supported by observations that the *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is selective for DA neurons of the SNpc in humans and primates, and yet can produce the full spectrum of motor signs seen in PD (96). Moreover, it is not even all of the SNpc DA neurons that appear to be involved in PD. The ventral-lateral tier is more severely affected than the dorsal tier (36), and this accounts for a more severe loss of DA in the putamen, where dorsally the loss can be as much as 95%, as compared to the caudate, where ventrally the loss can be as little as 60% (87). Some central dopaminergic systems, such as the ventral tegmental area and hypothalamic systems, are relatively spared, and descending spinal dopaminergic systems are spared entirely (6).

Although some DA neurons are spared in PD, it is also the case that neuron loss is not restricted to the dopaminergic neurons. Other catecholaminergic cell groups including the locus coeruleus are involved, as are some cells of the sympathoadrenal system and the serotonergic neurons of the raphe nuclei (6). There is also loss of cholinergic neurons

of the nucleus basalis of Meynert, and this may be responsible, at least in part, in some cases, for dementia (159).

Lewy Bodies

Another pathologic hallmark of PD is the Lewy body, an eosinophilic inclusion identified within neurons. On histologic stains, Lewy bodies have an eosinophilic core, and a surrounding pale halo. They are usually rounded, although their shape can be pleiomorphic (50), and they are generally 5 to 25 μm in diameter. They usually are observed within the cell soma, but also can be seen in neurites or free in the extracellular space. Lewy bodies are commonly observed in the brain regions showing the most neuron loss in PD, including SN, locus coeruleus, the dorsal motor nucleus of the vagus, and the nucleus basalis of Meynert, but they are also observed in neocortex, diencephalon, spinal cord, and even peripheral autonomic ganglia (50).

On ultrastructural analyses, Lewy bodies consist of an electron dense granular core and a peripheral halo consisting of radially oriented filaments 7 to 8 nm in width (28). The filaments resemble neurofilaments, and can be immunostained with antisera to neurofilament proteins (53), including the NF-L, -M, and -H forms (67). Immunostaining can be achieved with antibodies that recognize phosphorylated as well as nonphosphorylated epitopes (13,40). The cellular kinases responsible for the phosphorylation are not known. However, two candidates, Ca^{2+} /calmodulin-dependent protein kinase II (75) and cyclin-dependent kinase 5 (14, 98,118), have both been immunolocalized to Lewy bodies.

Another major antigenic feature of Lewy bodies is the expression of cellular proteins involved in protein degradation, including ubiquitin (93), and the proteasome (37,71). Presence of these antigens has been hypothesized to represent efforts on the part of the cell to degrade the abnormal protein aggregate.

Following the identification of mutations in the α -synuclein gene in a few cases of familial PD (see below), it was discovered that α -synuclein is a component of Lewy bodies (11,142,143). α -Synuclein in Lewy bodies can be labeled with antibodies to the C- or N-terminal, suggesting that full-length molecules are present (142). α -Synuclein immunostaining is identified in isolated Lewy filaments, and the pattern of staining suggests a polar orientation of the molecules (142). Staining of filaments *in situ* has been confirmed by immunoelectron microscopy (10). Synphilin, a protein known to interact with α -synuclein (33), also has been identified within Lewy bodies in PD (156).

α -Synuclein is identifiable not only in Lewy bodies of PD, but also the Lewy bodies of Hallervorden-Spatz syndrome (8), familial Alzheimer's disease (99), sporadic Alzheimer's (55), Alzheimer's associated with Down syndrome (100), and diffuse Lewy body disease (10,11). In Alzheimer's disease, Lewy bodies demonstrated by immunostaining for α -synuclein are most often observed in the amygdala.

The demonstrations of Lewy bodies in Alzheimer patients add to the growing evidence of important pathologic overlaps between Alzheimer's and PD. This evidence is further supported by the demonstration of co-localization of phosphorylated τ and α -synuclein in Lewy bodies of PD and diffuse Lewy body disease (9).

In addition to its localization in Lewy bodies in PD, abnormal α -synuclein immunostaining has been identified in axon terminals in the hippocampal dentate, hilar, and CA 2/3 regions in PD (44). Whereas immunostaining for β -synuclein has not been observed in Lewy bodies, staining was observed in these axon terminals. In addition, although immunostaining for γ -synuclein is not present in Lewy bodies, it is observed within axonal spheroids in the hippocampal dentate molecular layer in PD (44).

ETIOLOGIC FACTORS

Aging

The possible role of aging in the pathogenesis of PD is suggested by its usual occurrence in late middle age, and by marked increases in its prevalence at older ages (109). The possible contribution of age to the expression of the disease is further supported by early studies showing a loss with age of striatal DA (18) and DA of cells in the SN (113). However, whereas the gradual loss of striatal dopaminergic markers (88,138) and SNpc neurons (36) with age has recently been confirmed, the pattern and timing of these losses differ from what occurs in PD, indicating that aging itself is not likely to play a direct role in the degenerative process. For example, although the number of dopaminergic terminals appears to decrease with age, this takes place with a different temporal and spatial pattern than occurs in PD (138). The loss of SN neurons in aging is linear and predominantly in the dorsal tier of the SNpc, whereas in PD it is exponential and predominantly in the lateral ventral tier (36,138). In addition, the SN in PD contains numerous reactive microglia, which are much less frequent in age-matched control brains, indicating an active destructive process that is not present in the normal aged brain (111,112). Thus, whereas there is no question that increased age is a risk factor for PD, it remains unclear what precise role aging plays in pathogenesis.

Environmental Factors

Consideration of a role for environmental factors in the cause of PD was given major impetus with the discovery in 1983 that exposure to MPTP is capable of inducing parkinsonism in humans (96). The role of environmental factors was given additional weight by initial results of twin studies, as discussed below, which initially appeared to exclude any important role for genetic factors. The possible role of environmental factors has been addressed by a num-

ber of epidemiologic studies that have been well reviewed by others (97,148). Many of these studies have shown associations between rural residence, well-water drinking, or herbicide/pesticide exposure and the risk of developing PD (148). However, the precise role played by any specific compounds has remained elusive.

Genetic Factors

For many years, genetic factors were considered unlikely to play an important role in the pathogenesis of PD. This concept was based largely on twin studies conducted in the early 1980s that demonstrated a very low rate of concordance for the disease among identical twins (157) [reviewed by Duvoisin (29)]. Nevertheless, many investigators recognized that PD could occasionally be identified in families (52). The most important advances in PD research in recent years have been the identification of specific disease-causing mutations, making it possible for the first time to begin to explore pathogenesis at the molecular level. For this review, we focus on the best documented and most widely investigated genetic causes—those in α -synuclein and parkin.

Synuclein

After mapping a disease-causing gene locus to the 4q21-q23 region (130) in a large Italian kindred (52), Polymeropoulos and co-workers (131) identified a base pair change from G to A at position 209, which resulted in an Ala to Thr substitution at position 53 in α -synuclein in this family and three small Greek kindreds. Whereas initially there was a question as to whether this may represent a benign polymorphism, that possibility was soon dispelled by the discovery of a second disease-causing mutation, an Ala to Pro substitution at position 30, in an unrelated German kindred (92). The likely role of α -synuclein in the pathogenesis of PD was further supported by the discovery of α -synuclein in Lewy bodies of sporadic PD cases, as outlined above. One of the important aspects of the discovery of these mutations in α -synuclein was that they immediately suggested a possible pathogenetic mechanism, that of protein aggregation, because α -synuclein had been identified in Alzheimer plaques (154), and a central portion of α -synuclein had been shown to have the capacity to self-aggregate (56). The role of α -synuclein in the protein aggregation hypothesis for PD is discussed in the next section.

Little is known about the normal physiologic function of α -synuclein. Scheller's group (104) was the first to discover the compound, identifying it in *Torpedo* as a brain-specific synaptic terminal protein. These investigators subsequently demonstrated that the rat protein homologue is likewise expressed in nerve terminals (105). α -Synuclein messenger RNA (mRNA) is predominantly expressed in forebrain structures, such as hippocampus and cortex, but also in a few specific midbrain-brainstem nuclei, including

the SNpc, locus coeruleus, and dorsal motor nucleus (105), which, interestingly, are typically involved in PD. The human homologue of α -synuclein was independently identified by Ueda et al. (154) and Jakes et al. (78), and demonstrated by Irizarry and colleagues (76) to be expressed in nerve terminals, as is the case for rat. These investigators also showed by fractionation studies that α -synuclein appears to be loosely associated with synaptic vesicles, and this localization has been confirmed in rat brain by ultrastructural analysis (74). Jensen and his colleagues (82) have shown that α -synuclein binds to vesicles via its amino-terminal region, and that it is carried with vesicles by the fast component of axonal transport. Interestingly, the A30P mutation appears to abolish vesicle-binding activity.

What specific physiologic role α -synuclein and its homologues may play as vesicle-binding proteins remains a mystery. George and co-investigators (46) independently identified an avian homologue of α -synuclein, synelfin, as a gene upregulated in the song control circuit during a critical period of song learning, and suggested that it plays a role in neural plasticity (46). We have shown that α -synuclein mRNA and protein are upregulated in the SNpc following early developmental striatal target lesion (85). This lesion results in the induction of apoptotic death in some, but not all, developing dopaminergic neurons (102). However, in this model α -synuclein is not expressed in apoptotic profiles; it is exclusively upregulated in normal-appearing neurons, suggesting that it plays a role either in maintaining their viability, or, alternatively, in plastic change after viability is established. Vila and co-workers (155) reached a similar conclusion in a model of chronic MPTP toxicity. In support of the possibility that α -synuclein may play a role in a plasticity response in these injury models is the observation that α -synuclein mRNA in SN is up-regulated during the first 4 postnatal weeks, a period of maximal differentiation and synaptogenesis among DA neurons (85). What precise role α -synuclein plays in the development of DA neurons remains to be established. Remarkably, homozygous α -synuclein null mice have thus far shown no obvious abnormalities in numbers or morphology of DA neurons; density of striatal dopaminergic terminals; the number, morphology, or patch/matrix distribution of striatal neurons; or the ultrastructural appearance of striatal synaptic terminals (4). These animals, however, do exhibit an increased release of DA in a paired stimulus depression paradigm. In addition, they show diminished behavioral activation following administration of amphetamine (4).

In view of α -synuclein's ability to self-aggregate, there has been a tendency to assume that the mutations cause a toxic gain of function related to aggregation. It is important to keep in mind, however, that its function is unknown, and that a loss of function may relate to disease pathogenesis. In that regard, we have found that α -synuclein mRNA levels are diminished in the SNpc of patients with sporadic PD (120). Markopoulou and colleagues (103) have shown in a

large Greek family with the G209A mutation that there is diminished expression of the mutant allele in lymphoblastoid cell lines, and they suggest that the parkinsonian phenotype may arise from haploinsufficiency.

Parkin

Mutations in the parkin gene were first identified in Japanese families with a unique variant of parkinsonism (89). This form is inherited in an autosomal-recessive pattern, and typically begins at an early age; in the series of 17 patients studied by Ishikawa and Tsuji (72), the age ranged from 9 to 43 years, with a mean of 28. Many of the clinical features of patients with autosomal-recessive juvenile parkinsonism (ARJP) closely resemble those of idiopathic PD: tremor at rest, rigidity, bradykinesia, postural instability, gait freezing, and marked improvement with levodopa. However, there are differences between ARJP and idiopathic PD in addition to the age at onset. Patients with ARJP more often present with dystonia, show a marked clinical improvement after sleep, and often show hyperreflexia (72). In general, they do not show cognitive decline or autonomic failure, and the course is slowly progressive. The motor predominance of their clinical signs is in keeping with the pathologic findings, which indicate neuronal loss restricted to neurons of the SNpc and the locus coeruleus (146). Lewy bodies are not observed (146).

ARJP was found to map to the chromosome 6q25.2-27 region, and a marker for this region, D6S305, was found to be deleted in a single Japanese patient (89). Screening of complementary DNA (cDNA) libraries with a probe for a putative exon, which was also deleted in this patient, led to the identification of a sequence encoding an open reading frame for a 465 amino acid protein (89). The deduced amino acid sequence of this protein contains a ubiquitin homology domain at the N-terminal, and a ring-finger motif at the C-terminal. The gene encoding the protein is large [>500 kilobase (kb)], and contains 12 exons. Deletion mutations were identified in four other affected patients in three independent families, confirming the pathogenetic significance. A 4.5-kb mRNA transcript was identified in many human tissues, including brain. In brain, it is expressed in various regions, including the SN (89).

Subsequent molecular genetic analysis of 34 affected individuals from 18 unrelated Japanese families revealed four additional deletional mutations (64), bringing the total to six identified at that time (89). The deletions affected exon 3, exon 4, and exons 3 to 4, and a 1-base pair (bp) deletion in exon 5 resulted in a frameshift and an early stop. Further molecular analysis of non-Japanese families in Europe, revealed that in addition to deletion mutations, a variety of point mutations resulting in either truncation or missense could also cause the phenotype (3). In addition, this study identified patients with a late age of onset, up to 58 years

in one case, and indicated that in some instances the clinical phenotype was indistinguishable from idiopathic PD (3).

There is now growing recognition that mutations in parkin may cause what clinically resembles idiopathic PD. In an investigation of the scope of the molecular and clinical features in Europe, Lucking and co-workers (101) found that among 73 families with early onset (<45 years) of parkinsonism and affected family members, 49% had parkin mutations. Among early-onset patients without affected family members, 18% had mutations. The majority (77%) of these were younger than 20 years of age. Many of the patients with parkin mutations lacked the signs thought to be characteristic of ARJP such as dystonia and hyperreflexia, and were clinically difficult to distinguish from idiopathic PD. In all, 19 different rearrangements of exons mutations were identified, including multiplications as well as deletions, and there were 16 different point mutations (101).

The neurobiology of parkin is only beginning to be explored. By immunohistochemistry, the protein has been localized at the regional level to SN and locus coeruleus, and at the cellular level to the cytoplasm (139). Nuclear staining was not observed. Parkin has been shown to play a role in protein degradation as a ubiquitin-protein ligase (140). These findings suggest that abnormal accumulation of proteins or abnormal regulation of the half-life of normal cellular proteins may play a role in cell death.

PATHOGENETIC MECHANISMS

Free Radicals and Deficits in Energy Metabolism

The concept that free radical-mediated injury may underlie the neuronal degeneration that occurs in PD has been, and continues to be, the leading hypothesis for its pathogenesis. The free radical theory has been the subject of many excellent reviews (34,122), so it will be outlined here only briefly. This theory is also referred to as the oxidant stress hypothesis or the endogenous toxin hypothesis. In their review, Fahn and Cohen (34) point out that the free radical hypothesis is appealing because four aspects of the neurochemistry of DA neurons and their local environment within the SN make the concept plausible. First, a major degradative pathway for DA is its oxidative deamination by monoamine oxidases A and B. This process results in the enzymatic production of H_2O_2 , which, while itself not a free radical, can nevertheless react nonenzymatically with ferrous or cupric ions via Fenton-type reactions to form highly reactive hydroxyl radicals. Second, DA can react nonenzymatically with oxygen to form quinones and semiquinones, with the production of superoxide, hydrogen peroxide, and hydroxyl radicals. Third, the SN, particularly the SN pars reticulata, is rich in iron, which as mentioned above, may in its ferrous state catalyze the formation of hydroxyl radicals from H_2O_2 . Fourth, the SN contains neuromelanin formed from the

auto-oxidation of DA. This auto-oxidation generates toxic quinones and reactive oxygen species. In addition, the presence of neuromelanin in the cell may alter the ability of metal ions to participate in the production of reactive oxygen species (145).

The possibility that DA neurons may undergo free radical-mediated injury in PD has received support from animal studies using one of two neurotoxins that can be used to selectively destroy DA neurons—6-hydroxydopamine (6-OHDA) and MPTP. 6-OHDA reacts with oxygen to produce superoxide anion radical, H_2O_2 , and hydroxyl radical. It is a general cytotoxin but derives its specificity by virtue of its affinity for the high-affinity catecholamine transporters. Thus, when used in sufficiently low concentrations, its actions can be directed toward catecholamine neurons. Moreover, it can be limited to acting on DA neurons by pretreating animals with an inhibitor of high-affinity norepinephrine uptake, such as desipramine (19,66,166).

Interestingly, like 6-OHDA, DA itself is a selective neurotoxin for DA neurons (38,54,61,62,114,135). This seems to be in large part due to its ability to oxidize to form reactive oxygen species, including DA quinone, which has a high affinity for the cysteinyl residues on proteins (41–43, 54,63). Thus, it seems possible that DA itself can be a source of oxidative stress, particularly under conditions of increased DA turnover and decreased antioxidant defenses (see below).

MPTP acts via its active product MPP^+ , which is selectively taken up into DA neurons via the DA transporter, and inhibits complex I activity in mitochondria. Inhibition of complex I not only interferes with adenosine triphosphate (ATP) synthesis, but also results in augmented production of superoxide anion radical. The possible role of superoxide radical in MPTP toxicity has received direct support by the demonstration by Przedborski and co-workers (132) that transgenic mice with high Cu/Zn superoxide dismutase activity are resistant to MPTP.

The free radical hypothesis of PD has also received support from studies of human postmortem brain. Free radicals can cause injury to cells by damaging DNA, proteins, and lipids of the cell membrane. There is evidence from postmortem studies for free radical-induced modification of each of these classes of molecules. Dexter and co-workers (24) have shown that in PD brain there is a reduction in levels of polyunsaturated fatty acids, which provide an index of the amount of substrate available for lipid peroxidation, and an increase in levels of malondialdehyde, an intermediate in the lipid peroxidation process. The increase in malondialdehyde was regionally specific for the SN. These workers subsequently confirmed evidence for abnormal lipid peroxidation in PD by identifying a tenfold increase in cholesterol lipid hydroperoxide, an early marker in the lipid peroxidation process (25). Free radicals are also capable of directly damaging DNA. Sanchez-Ramos and colleagues (136) have shown that regional concentrations of 8-hydroxy-deoxygua-

nosine, an index of oxyradical-mediated DNA damage, are increased in the caudate and SN of PD patients. Relatively less attention has been given to the possibilities of oxygen-mediated damage to proteins, or of advanced glycosylation changes to proteins in PD (141). The possibility that such protein changes may also occur in PD brain is supported by the demonstration that protein adducts of 4-hydroxy-2-nonenal, a cytotoxic product of lipid peroxidation, can be identified by immunohistochemistry in many nigral neurons of PD patients in contrast to age-matched controls (162).

Postmortem studies have also revealed neurochemical features that may predispose the PD brain to oxidative damage. Reduced glutathione is an important endogenous antioxidant, and it has been reported to be reduced in the SN in PD (127). Jenner and colleagues (80) have confirmed low levels of reduced glutathione in the SN of PD patients, and have shown that the alteration is disease-specific. Interestingly, they have also shown that reductions are observed in patients with incidental Lewy body disease, which may be a preclinical form of PD (49). This finding suggests that the reduced levels of glutathione may be a fundamental and primary abnormality in PD, rather than a secondary change.

A number of postmortem studies have also suggested that abnormalities of iron metabolism may underlie the neurodegeneration of PD. Iron metabolism is of particular interest in relation to the free radical hypothesis because, as noted above, it normally is found in high concentrations in SN, and is capable of catalyzing free radical formation. Dexter and colleagues (26) reported increased levels of iron in the SNpc of PD patients. This observation took on potentially greater significance when this group subsequently reported decreased levels of ferritin in PD brains (23), as ferritin normally sequesters iron in an unreactive state. However, it has become apparent that increased iron levels may be observed in many brain regions demonstrating neural degeneration in a variety of diseases of the basal ganglia (22), so the specificity of changes in iron levels in PD is less clear. Nevertheless, the possible relationship of altered iron metabolism to the pathogenesis of PD remains of interest, based on the finding of a higher density of lactoferrin receptors on neurons and microvessels of patients with PD (35). This finding suggests that lactoferrin receptors, which regulate intraneuron iron content, may be overexpressed in vulnerable dopaminergic neurons in PD.

Another postmortem finding in PD patients that is compatible with the free radical hypothesis is that of a deficiency in mitochondrial complex I. Such a defect could either result in the abnormal production of free radicals, or be the result of free radical injury (137). This defect takes on particular interest in light of the observation that MPP^+ , the toxic oxidative product of MPTP, inhibits complex I (121). The defect in complex I in PD patients has been demonstrated by Schapira et al. (137) to result in a mean 37% decrease in activity. This decrease appears to be both regionally spe-

cific for the SN, and disease specific, among basal ganglia disorders, for PD.

Thus, the free radical hypothesis receives indirect support from a large number of separate lines of evidence, and, as stated above, it remains the foremost and most widely tested hypothesis of neural degeneration in PD. Nevertheless, it remains only a hypothesis, and it has its shortcomings (17). For example, there is no specific aspect of the free radical hypothesis as it is currently posed to account for the relative vulnerability of ventral tier dopaminergic neurons in PD. In addition, it must be remembered that nonaminergic neuronal groups, such as the nucleus basalis, which is cholinergic, also degenerate in PD, and aspects of the free radical hypothesis that are dependent on catecholamine metabolism are not relevant to the degeneration of these structures.

Programmed Cell Death

The concept that a genetically regulated cell death process may underlie the neuron-specific degenerations of later life has gathered great attention in recent years. The programmed cell death hypothesis in fact may be related to the concept of free radical-mediated cell death. Although traditional concepts of free radical injury have centered on the ability of toxic molecules to directly injure cellular constituents without any participation of the host cell's own genetic programs, it is now clear that in both *in vitro* and *in vivo* paradigms of free radical-mediated injury programmed cell death may occur. It is also apparent that in some settings programmed cell death may be carried out by the controlled production of free radicals.

In relation to PD, it is important first to consider the evidence that programmed cell death does in fact occur within dopaminergic neurons. As predicted by classic neurotrophic theory, some natural cell event does occur during development in the SNpc, with typical light microscopic morphology of apoptosis, demonstrated both by Nissl stain and suppressed silver staining (79), and we used a double-labeling technique to identify apoptotic natural cell death in phenotypically defined dopaminergic neurons (123). Natural cell death in these neurons has a bimodal time course. There is an initial, major peak that begins on embryonic day 20, and largely abates by the eighth postnatal day (PND). There is a second, minor peak of natural cell death on PND 14. The presence of a postnatal cell death event is in keeping with the demonstration by Tepper and colleagues (151) that there is a decrement in the number of TH-positive neurons in SN postnatally, particularly in the first postnatal week. Although there is evidence that the magnitude of the natural cell death event in DA neurons is regulated by interactions with the target striatum (see below), as classic neurotrophic theory would predict, it remains unknown which neurotrophic factors are involved. We have shown *in vitro* in postnatal primary cultures of DA neurons that GDNF is uniquely able to support viability by

suppressing spontaneous apoptotic death (16) as well as cell death initiated by 6-OHDA (see below). Whether glial cell line-derived neurotrophic factor (GDNF) plays such a role *in vivo* remains to be determined.

We have shown that natural cell death in SNpc can be regulated during development by striatal target interactions. Excitotoxic injury to the striatum on PND 7 results in an eightfold increase in the number of apoptotic profiles (102). These profiles are morphologically identical to those observed during natural cell death, and meet ultrastructural and 3' end-labeling criteria for apoptosis. Within SNpc, induction of cell death is identified within phenotypically defined dopaminergic neurons.

Programmed cell death also occurs in DA neurons in animal models of parkinsonism. Intrastriatal injection of 6-OHDA results in an induction of apoptotic death in phenotypically defined DA neurons of the SN (106). This induction is most pronounced in a developmental setting, through PND 14, but it can also be demonstrated, at a lower level, in mature animals. Interestingly, at older ages the morphology of cell death becomes mixed, including apoptotic and nonapoptotic features (106). Although in this model 6-OHDA may lead to apoptotic death simply by the destruction of terminals and the resulting failure of target support, there is evidence that the toxin also directly mediates death. In addition to the fact that 6-OHDA is able to induce death long after it is possible to demonstrate target dependence (84), the morphologic features of activated caspase-3 expression, an important mediator of programmed cell death, differs in 6-OHDA-induced death, as compared to natural and target injury-induced cell death (83).

Other important animal models of parkinsonism, in rodents and primates, are induced by MPTP, or its active oxidized product, MPP⁺. A number of investigators have shown in a variety of systems that MPP⁺ can induce apoptosis *in vitro*. Dipasquale and colleagues (27) first showed that MPP⁺ can induce apoptotic morphology and DNA fragmentation in postnatal cerebellar granule cells in culture. Subsequently, others have shown that MPP⁺ appears to induce apoptosis in embryonic mesencephalon culture (116), in PC12 cells, both differentiated (117) and undifferentiated (57), and in a human neuroblastoma cell line (73). Tatton and Kish (149) have shown that when MPTP is administered to mice in low doses over a 5-day course, it induces apoptotic death. In this model, induction of apoptosis is dependent on the dosing regimen; acute administration of multiple doses in a single day results in non-apoptotic death (77). Further support for the role of programmed cell death in the MPTP mouse model derives from the observation that Bax, a mediator of apoptosis, is induced (60). In addition, overexpression of Bcl-2, a protein inhibitor of apoptosis, diminishes MPTP-induced injury (161).

It remains controversial whether apoptosis can be identified in the Parkinson brain. One initial report demonstrated intranuclear chromatin clumps by electron microscopy (7),

but they were not clearly characteristic of apoptosis. TUNEL labeling (45) has been demonstrated in PD brains (115). However, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique is not entirely specific for apoptosis since it can also label free 3'-ends that are generated by necrotic death, producing false positives. Thus, it is essential to co-identify not only TUNEL labeling but also the classic chromatin clump morphology of apoptosis. Tompkins and co-workers (153) and Tatton and colleagues (150) have both achieved this more specific demonstration. However, many other investigators have been unable to identify specific TUNEL labeling in PD brains (12,86,91,160). Thus, a consensus has not been achieved, and further investigation will require methods beyond these morphologic techniques, such as the utilization of specific biochemical markers for programmed cell death. Hartmann and colleagues (58) have made such an effort utilizing an antibody that is specific for the activated form of caspase-3. They have shown that activated caspase-3 could be identified in Lewy body-containing neurons of the SN in PD brains (58). However, activated caspase-3 was also identified in control brains, in larger numbers of neurons. They attribute this staining to agonal changes. Thus, the staining was not specific for PD, and more study is needed with additional specific immunoreagents for other components and by-products of programmed cell death pathways.

Protein Aggregation

The possibility that protein aggregation may play a role in PD had long been suggested by the presence of Lewy bodies in disease brains. However, this concept was given powerful support upon discovery of the mutations in α -synuclein. Human α -synuclein was originally identified as a proteolytic fragment derived from Alzheimer senile plaques (154). The isolated fragment, termed the non-A β component of amyloid (or NAC), corresponded to a 35 amino acid hydrophobic portion of α -synuclein. Soon after its discovery, NAC was predicted to form β -sheet secondary structure, and shown to self-aggregate to form fibrillar amyloid *in vitro* (56). Thus, with the discovery of α -synuclein, PD was placed firmly among neurodegenerative disorders for which protein aggregation is believed to play an important pathogenic role, including Alzheimer's, motor neuron disease, the triplet repeat diseases, and the prion diseases.

Subsequent biochemical studies have shown that full-length α -synuclein is capable of binding to A β 1-38 and forming amyloid (163). This binding requires the hydrophobic NAC region. Other investigators have confirmed α -synuclein binding to A β , and have shown that α -synuclein is capable of homodimerization (81,124). Even in the absence of A β , full-length recombinant α -synuclein is capable of self-aggregation *in vitro* to form fibrillar amyloid material (59). A number of investigators have confirmed this observation, and have found that one or both mutant forms are more likely to form β -sheet and aggregate, forming fibrils

that resemble those of Lewy bodies (20,32,47,119). Crowther and co-workers (21) have shown that C-terminally truncated α -synuclein more readily self-assembles into filaments that resemble those isolated from diseased brain.

Further analysis of the NAC fragment has shown that the N-terminal sequence is responsible for aggregation (30). NAC aggregates have been demonstrated to have cellular toxicity. They induce apoptotic death in cultured human neuroblastoma cells (31). Low concentrations of aggregated NAC are toxic to DA neurons in primary culture and neuronally differentiated PC12 cells (39). *In vivo* application of NAC aggregates induced death of SN DA neurons. Based on these demonstrations of the ability of α -synuclein and NAC to aggregate and the possible toxicity of aggregates, an abnormality of protein aggregation has become one of the principal hypotheses for the pathogenesis of PD.

FINAL COMMENT

Although Parkinson's disease was first described almost two centuries ago, it is only recently that we have begun to understand the complex nature of the functional deficits that it entails or its neurobiological causes. Yet, the pace of discovery is quickening. With the discovery of the genetic basis of some familiar forms of the disorder, the appreciation of trophic factors that influence DA neurons, and the development of new technologies such as the use of stem cells and viral vectors, there is every reason to believe that within a generation Parkinson's disease will become a chapter in the history of diseases of the past.

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