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Neurochemistry of Nigral Degeneration

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INTRODUCTION

Progressive degeneration of the dopaminergic neurons of the substantia nigra is the pathological hallmark of idiopathic Parkinson's disease (PD). Based on cytoarchitectonics and melanization, Hassler divided the human substantia nigra into many subsections and demonstrated that the ventral and lateral regions of the substantia nigra may be preferentially involved in early stages of the disease (1,2) (Fig. 1), an observation that was later confirmed in human PD (3,4). The etiology of the progressive degeneration of the substantia nigra pars compacta cells is unknown. It has been proposed that the clinical signs and symptoms of PD emerge only after a loss of 75% of the nigral neurons. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies also indicate that the rate of loss of dopaminergic neurons is about 6–13% in patients with PD compared to 0–2.5% in healthy controls (5). These facts suggest that the process of degeneration of nigral neurons is initiated several years ahead of the onset of the clinical expression of the disease.

Epidemiological evidence emphasizes the role of environmental toxins in the development of PD. Discoveries of gene mutations responsible for

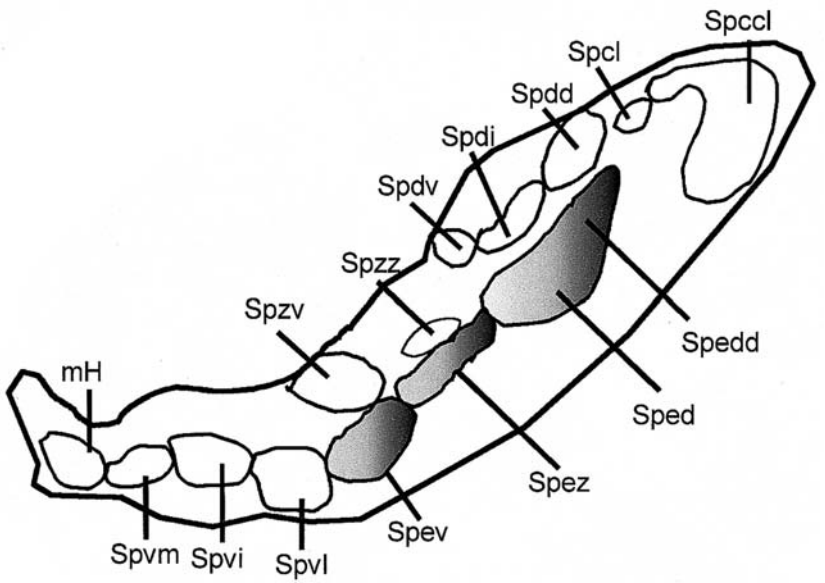

FIGURE 1 A diagrammatic representation of the subdivisions of human substantia nigra as defined by Hassler. The shaded subnuclei in the ventral and lateral regions degenerate selectively in the early stages of PD. (Adapted from Refs. 1, 2.)

inherited forms of PD have increased interest in the role of genetics in the etiology of PD. Modern molecular biological approaches are pointing to the possibility that dysfunction of a variety of cellular mechanisms may result in an insidious and a slowly progressive levodopa-responsive parkinsonism that is indistinguishable from PD. These observations raise the issue of whether there may be multiple etiologies for PD. In this chapter, some of the neurochemical changes noted in the degenerating dopaminergic neurons of the substantia nigra in experimental models of PD, inherited forms of PD, and idiopathic PD will be summarized.

GROWTH FACTORS AND THE SUBSTANTIA NIGRA

Neurons and glia need a very small concentration of trophic or growth factor(s) for their continued existence, maintenance of a normal connectivity and physiological state, as well as recovery from chemical and physical injury (6). If these growth factors are withdrawn or if their continuing influence is affected both neurons and glia may fail to differentiate and die during embryogenesis. As adult neurons, they may lose their efficiency or

become vulnerable to toxic factors and ultimately die. Under stressful circumstances, neurons and glia upregulate the expression of growth factors and their specific receptors or acquire them from their target neurons and the surrounding glial cells and recover from the injury. Age-dependent decline and withdrawal of neurotrophins and their receptors (7,8) are thought to play an important role in the pathogenesis of Alzheimer's disease. The dopaminergic neurons of the substantia nigra are dependant on several such trophic and growth factors for normal ontogenesis and survival as fully differentiated mature adult neurons. In experimental models of PD and in idiopathic PD, the dopamine neurons of the substantia nigra show a deficiency of expression of several growth factors. It is possible that withdrawal of these growth factors in the dopaminergic neurons of the substantia nigra may contribute to the pathogenesis of idiopathic PD.

Trophic Factors and Ontogenesis of Dopaminergic Neurons of the Substantia Nigra

The normal development of dopaminergic neurons in ventral mesencephalon depends on the influence of many trophic factors. Between embryonic days E9.5 and E16, several trophic factors play important roles in the induction, differentiation, as well as complete maturation of dopamine synthesis, release, and reuptake machinery of ventral mesencephalic dopamine neurons (Fig. 2). Around the embryonic day of E 9.5, two such factors, namely sonic hedgehog (SHH) (9) and fibroblast growth factor 8 (FGF8), define the site of induction of dopamine neurons in the ventral mesencephalon. SHH is a member of the hedgehog family of signaling protein that plays a major role in the differentiation of diverse groups of neurons in the ventral half of the neural tube, including dopamine and serotoninergic neurons (10). SHH may even play an important role in maintenance of adult dopamine neurons since intrastriatal injection of SHH diminishes the motor behavioral defects of 6-hydroxydopamine (6-OHDA) models of PD (11).

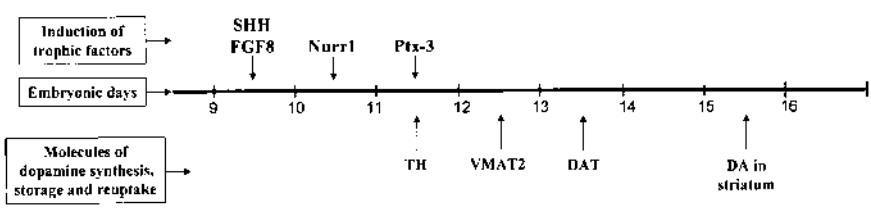

FIGURE 2 The ontogenesis of dopaminergic neurons of substantia nigra in rats and mice. (Adapted from Refs. 9, 12, 26, 27, 28.)

Around embryonic day E10.5, Nurrl is expressed in a group of cells in the ventral mesencephalic region (12). Nurrl is a member of the "orphan receptors" transcription factors and is expressed in several areas of the brain, but it is expressed intensely and selectively in the dopaminergic neurons of the ventral mesencephalon (12,13). Nurrl knockout results in the complete lack of development of dopaminergic neurons in the midbrain (14,15) with almost 98% reduction of dopamine in the striatum (16). Nurrl and tyrosine hydroxylase (TH) are coexpressed, with the maximum intensity in the midbrain substantia nigra neurons (17–19). The expression of dopamine transporter molecule may also be dependant on the Nurrl gene (20). Nurrl is expressed even in adult neurons, suggesting a role for a continuing influence on TH expression and survival of adult nigral dopamine neurons (21). Nurrl-deficient animals are more susceptible to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment (22), and Nurrl polymorphism may be associated with familial and idiopathic PD (23).

One day after expression of Nurrl, on E11.5, another factor, Pentraxin 3 (Ptx3), is expressed solely in the dopaminergic neurons of the ventral tegmental area and the substantia nigra (24). Ptx3 is reduced in the 6-OHDA model of PD as well as in idiopathic PD (24). The expression of Ptx3 in these neurons coincides with the expression of the TH gene in the same neurons (25). The appearance of Nurrl and Ptx3 early in the ontogenesis of dopaminergic neurons suggests that these factors are responsible for differentiation and maturation of ventral mesencephalic dopamine neurons rather than establishment of connectivity to the striatum. The expression and maturation of other components of dopamine release, reuptake, and storage are completed by the embryonic day E16, and the release of dopamine in the striatum is first noted around E17 (26–28).

Besides SHH, Nurr-1, and Ptx-3, NFIAb (29), LMX1b (30), heparin-binding epidermal growth factor-like growth factor (HB-EGF) (31), En-1, and En-2 of the engrailed gene family (32) have roles in the survival of the midbrain dopaminergic system during ontogenesis. En-1 and En-2 also appear to regulate the expression of α-synuclein, the major constituent of Lewy bodies in PD (32).

Growth Factors and Nigral Injury

Besides these trophic factors that play important roles in the ontogenesis of nigral dopamine neurons as well as maintaining the survival of differentiated adult dopamine neurons in the nigra, several other neurotrophic factors may play a neuroprotective role in many models of injury to the dopaminergic neurons of the substantia nigra (8,33). These neurotrophic

factors have significant structural and functional similarities and are classified into the nerve-growth factor (NGF) superfamily, the glial-derived neurotrophic factor (GDNF) family, the neurokine family, and the nonneuronal growth factors.

The superfamily of neurotrophins includes the NGF, brain-derived neurotrophic factor (BDNF), and neurotrophins (NT) 3, 4/5, and 6. All members of the neurotrophin family interact with two structurally unrelated receptors, namely the tyrosine kinases (*trk*A, *trk*B, and *trk*C) and the low-affinity binding neurotrophin receptor $p75^{NTR}$ (34,35). Recent studies have shown that the *trk* and $p75^{NTR}$ are coexpressed in the same cell and that the molecular signaling pathways of these two receptors may be stimulated independently or coactivated (36,37). Even though stimulation of these two pathways may interact with each other, they may have opposite effects. Stimulation of the *trk* receptors subserves a neuroprotective effect by inactivating several factors that are apoptotic (35). The $p75^{NTR}$ is structurally unlike the *trk* receptors. It belongs to the tumor necrosis factor (TNF) receptor family and contains the "death domain" (35,38). Stimulation of $p75^{NTR}$ has been shown to induce several molecules that can initiate apoptosis, but under other conditions $p75^{NTR}$ stimulation can be antiapoptotic (34,35).

The dopaminergic neurons of the substantia nigra in humans immunostain for proteins of FGF, BDNF, and NT3 as well as *trk*A, *trk*B, and *trk*C. The immunoreactivity is noted more intensely in the medial areas of the nigra than the lateral areas (39), providing one piece of evidence for selective loss of neurons in the lateral regions. BDNF, when injected into the striatum, is transported only to the soma of dopaminergic neurons of the substantia nigra. These findings suggest that BDNF found in nigral neurons may be synthesized locally within the neurons or can be acquired by retrograde transport from the striatal neurons (40). The melanized dopamine-containing neurons of the substantia nigra show a greater loss of BDNF than the nonmelanized cells (41,42). BDNF prevents nigral degeneration induced in MPTP models of PD (43).

GDNF, a neurotrophic factor belonging to the TGF-β superfamily, and its receptors, Ret and GDNF-α1, are expressed highly in the dopaminergic neurons of the substantia nigra (8,33). GDNF concentrations are decreased in the nigral neurons in PD (44). When injected in the striatum, GDNF is selectively transported retrogradely to nigral dopaminergic neurons (40). GDNF, like BDNF, protects mesencephalic dopamine neurons from 6-OHDA and MPTP toxicity and improves motor functions in these models of PD (45,46). However, the molecular pathways that mediate neuroprotective effects of these two neurotrophic factors may be different (47).

Neurokines are neuropoietic cytokines. Among the neurokines, an increased level of interleukin-6 (IL-6) has been demonstrated in the striatum. Many cytokines, which are traditionally recognized to play anti-inflammatory roles outside the brain, have now been recognized to be expressed in the brain (48) in response to tissue injury or inflammation (e.g., multiple sclerosis) (49). Some of these cytokines may be neuroprotective and others apoptotic. In this regard, levels of TNF-α, TGF-α, TGF-β, IL-1β, IL-4, and IL-6 are elevated in the striatum in PD. This has led to the hypothesis that the pathogenesis of PD may be the result of an imbalance between the actions of the antiapoptotic neurotrophic factors and the proapoptotic factors (50,51).

Several neurotrophic factors have been recognized to be expressed by nonneural cells. Among these, basic fibroblast growth factor (bFGF or FGF2) has been demonstrated to be expressed in the substantia nigra (52), and a profound depletion of bFGF was noted in the nigral neurons in PD (53,54). Acute and intermittent injections of nicotine increase FGF2 expression and mediate neuroprotective effects in several models of neuronal injury (55,56) including 6-OHDA and MPTP models of PD (57–59).

Summary

It is important to point out that it is unclear whether the reduction of the neurotrophic factors noted in the nigral neurons of PD is the cause or a consequence of nigral degeneration. While in animal models of acute injury the striatum and the dopaminergic neurons of the substantia nigra may be protected by these neurotrophic factors, the role played by these factors in PD remains to be established. Even if the observed decrease in neurotrophic factors and their receptors is a consequence of the disease itself and not the cause, reintroduction of these trophic factors using viral vectors or drugs that will inhibit or activate the different molecules involved in the pro- and antiapoptotic pathways, respectively, will be an important mode of therapy for PD, Alzheimer's disease, and other neurodegenerative disorders in the future. Among these neurotrophic factors, at the present time BDNF, GDNF, and FGF2 appear to show the greatest promise.

DYSREGULATION OF PROTEIN METABOLISM

The identification of gene mutations that are responsible for causing inherited forms of PD has expanded our focus from environmental causes of PD to the possible role of genetics in the etiology of PD. Several studies demonstrate that mutations of genes result in mutant proteins that are

inefficiently catabolized by the protein removal system, the ubiquitin-proteasome pathway. A defective ubiquitin-proteasome pathway can also be the result of gene defects. Failure of the ubiquitin-proteasome system to degrade a protein because it is unable to recognize the mutant protein or due to an inefficiency of the ubiquitin-proteasome system itself will result in aggregation of the mutant proteins within nigral cells and cause neurotoxicity. Evidence for both types of abnormalities has been observed in inherited forms of PD. Dysfunction of the ubiquitin-proteasome system could potentially be an important factor in the pathogenesis of PD.

Mutant Proteins and Inherited Forms of PD

An alanine-to-threonine substitution at codon 53 (A53T) of the gene for α-synuclein has been identified in several families with Italian-Greek pedigree (60). A substitution of proline for alanine at codon 30 (A30P) of the α-synuclein gene was also described in a family of German pedigree (61). The mRNA of α-synuclein is expressed throughout the brain, but expressed at a very low intensity in the substantia nigra, and the level of expression of mRNA for α-synuclein is much lower than normal in the nigral neurons of PD brains (62). The protein of α-synuclein localizes to both the nucleus and the synapse, but its function is predominantly presynaptic (63). α-Synuclein has structural similarities to the chaperone protein 14-3-3 (64), and together, α-synuclein and 14-3-3 regulate the expression of TH (65).

Recognition that mutations of the Parkin gene are responsible for autosomal recessive-juvenile parkinsonism (AR-JP) is a major breakthrough in our understanding of the pathogenesis of PD (66). The Parkin gene maps to chromosome 6q25.2-q27 and has 12 exons coding for a 465-amino-acid protein. Mutations of the Parkin gene are the most common type noted in autosomal recessive PD (66). Parkin is an E3 ubiquitin ligase (67), an enzyme that plays an important role in the ubiquitin-proteasome protein degradation pathway. The E3 ubiquitin ligase family consists of a large number of members and among these, Parkin is the type that contains, within the same molecule, a ring finger domain that binds to ubiquitin as well as a site that recognizes and binds to the substrate (68).

α-Synuclein (66), alphaSp22, which is a glycosylated isoform of α-synuclein (69), CDCrel-1 (67), a synaptic vesicle–associated protein, synphilin-1 (70), Pael receptor (Parkin-associated endothelin receptor-like receptor) (71), and a G-protein–coupled transmembrane polypeptide that is expressed most intensely in the TH-positive neurons of the nigra (72) are some of the substrates that interact with Parkin.

A mutation of the gene that codes for ubiquitin carboxy-terminal hydrolase (UCH-L1) has been recognized in one family with an inherited

form of PD (73). Ubiquitin hydrolases are deubiquitinating enzymes that play a pivotal role in maintaining a steady-state level of ubiquitin by generating and recycling ubiquitin (68). A mutation of the human neurofilament M gene has also been reported in a patient with young-onset PD with a French-Canadian pedigree (74).

Ubiquitin-Proteasome Protein Degradation Pathway and PD

Ubiquitin-proteasome–mediated protein catabolic pathway plays a major role in maintaining a viable and normal functioning cell. Dysfunction of the ubiquitin-proteasome pathway has been proposed to be involved in many neurodegenerative disorders, including Alzheimer's disease, frontotemporal dementia, Huntington's disease, and several types of malignancies (68,75). The two basic mechanisms that are involved in the catabolism of proteins by the ubiquitin-proteasome pathway are (1) the protein that needs to be degraded is tagged with ubiquitin and (2) the tagged protein is then transferred to a protease, 26S proteasome, which degrades the tagged protein into small peptides. The initial step for ubiquitination of the substrate consists of activation of the inactive form of ubiquitin by the ubiquitin-activating enzyme E1. The activated ubiquitin is then transferred to the ubiquitin carrier protein family of enzymes (ubiquitin conjugating enzyme or Ubc) E2. Among the 13 subtypes of E2, UbcH7 and UbcH8 appear to play a more prominent role in interacting with the subtype of E3 ubiquitin ligase implicated in AR-JP (66). E3 (ubiquitin ligase), consisting of a large family of ligases, is responsible for recognizing the substrate protein that is to be degraded and facilitating the transfer of activated ubiquitin from E2 so that the substrate can be tagged with ubiquitin and subsequently recognized by the proteasome. This substrate-ubiquitin complex is further polyubiquitinated by a polyubiquitinating enzyme E4 and presented to the 26S proteasome. The proteasome then degrades the tagged protein to small polypeptides, and peptidases further degrade the peptides into amino acids. Ubiquitin is released for further use by one of several deubiquitinating enzymes. The deubiquitinating enzymes play the important role of regulating the amount of ubiquitin available (Fig. 3).

Gene defects resulting in dysfunction of many of the different molecules involved in the ubiquitin-proteasome pathway have been shown to induce PD. These mutations of the genes may result in (1) mutant forms of ubiquitin-domain proteins (UDPs), which will decrease the availability of free UDPs; (2) mutant ubiquitin ligase (E3), which will result in a failure of E3 to recognize the substrate; (3) mutations of the protein substrate; or (4) a failure to deubiquitinate, which will result in a decreased supply of free ubiquitin to inadequate recycling of ubiquitin. The N-terminal region of the

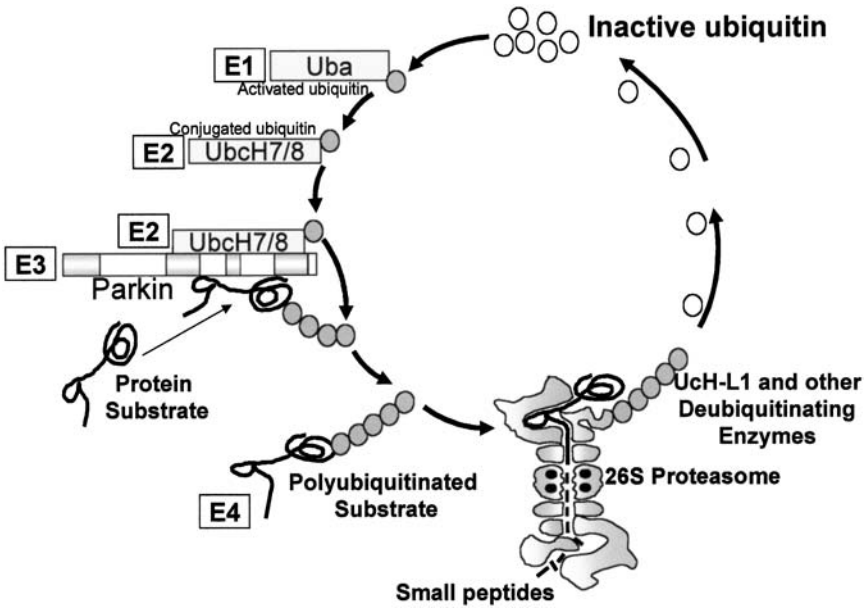

FIGURE 3 Diagrammatic representation of the Parkin-Ubiquitin proteasome pathway. See text for details. (Adapted from Refs. 66, 67, 68, 72.)

Parkin protein codes for an ubiquitin-domain protein (UDP). The UDPs have structural similarities to ubiquitin and function as proteasome adapters (76). A mutation of the ubiquitin-like domain coding regions of exons 2 and 3 of the Parkin gene alone has been observed in AR-JP (66). Deletion, duplication, and mutations of several regions of the Parkin gene have been recognized to cause AR-JP. Among these, mutations in the regions coding for ring fingers appear to be quite frequent among AR-JP patients (66). Mutation of α-synuclein may result in failure of the ubiquitinating system to recognize the substrate. Even though only two members of the family were reported to have mutations of the gene of UCH-L1, resulting in only a partial suppression of the deubiqutinating enzyme UCH-L2, these two patients reinforce the concept that disturbances of the protein degradation system will lead to aggregation of protein within the neuron. Proteasomal dysfunctions have also been observed in PD (77).

Neurochemistry of the Lewy Body

Mutated and misfolded proteins tend to aggregate. During aggregation, as commonly seen in polyglutamine repeat diseases, these fibrillary proteins

may also sequester other proteins, including chaperone proteins and ubiquitin-conjugating enzymes, and further contribute to prevention of catabolism of these mutant proteins (78). The presence of the Lewy body, an example of such protein aggregation, in the substantia nigra is considered pathognomonic of PD. Electron microscopically, the Lewy body consists of a dense circular central core surrounded by neurofilaments located in the pale halo at the periphery (79). Aggregated α-synuclein is a major constituent of Lewy bodies in PD. The central core as well as the peripheral halo immunostain very strongly for the full length of α-synuclein (80). β or γ-Synuclein appears to aggregate in the axon terminals of hippocampal neurons of PD and diffuse Lewy body brains (81).

An overexpression of α-synuclein, as demonstrated in *Drosophila* models of PD (82), is neurotoxic to nigral neurons, but this toxicity can be reversed by overexpression of two other chaperone proteins, namely HSP70 and HSP40 (83). In the presence of iron, which accumulates in the ventral mesencephalon in PD, or of aluminum, copper, or manganese ions, α-synuclein appears to be insoluble (84). Increased oxidative stress can be an early step for aggregation of α-synuclein (85), and in turn, aggregation of α-synuclein can promote further mitochondrial dysfunction and oxidative stress (86). α-Synuclein accumulation can decrease the effectiveness of proteasomal function and contribute to the accumulation of other proteins that are substrates for proteasomal degradation. Understanding the mechanisms of insolubility of α-synuclein is crucial to creating new modes of therapy for PD and other synucleinopathies.

Besides α-synuclein, Lewy bodies also contain several other proteins. These proteins can be broadly grouped into several types, namely (1) proteins that have presynaptic functions, (2) neurofilaments and related proteins, (3) markers of oxidative stress, (4) pro- and antiapoptotic proteins, (5) molecular chaperones, and (6) members of the ubiquitin-proteasome system (Fig. 4).

Summary

The discovery that mutations of the α-synuclein and Parkin genes cause a parkinsonian syndrome has led to a better understanding of the mechanisms with which the dopaminergic neurons of the substantia nigra handle protein degradation. The evidence indicates that in PD, as in several other synucleinopathies, the aggregation of α-synuclein and ubiquitin in Lewy bodies is the result of ineffective removal of proteins by the ubiquitin-proteasomal system of the dopaminergic neurons of the substantia nigra.

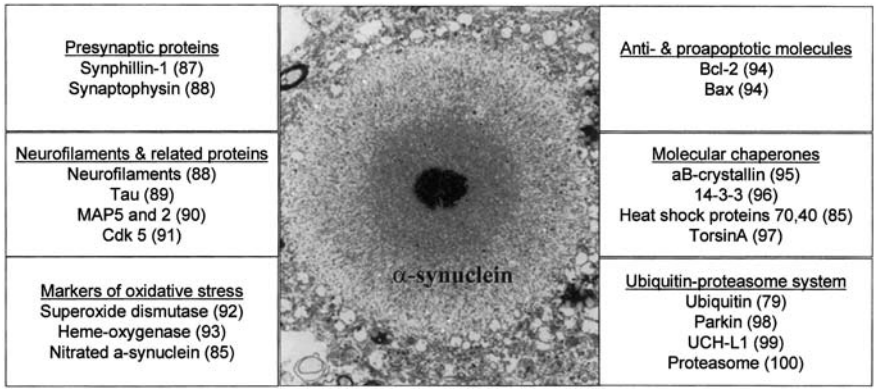

FIGURE 4 Diagrammatic representation of a Lewy body. The different proteins that are associated with Lewy bodies in PD are listed in boxes.

MITOCHONDRIAL DAMAGE AND THE SUBSTANTIA NIGRA

The concept that mitochondrial dysfunction can cause a parkinsonian syndrome came into focus with the observation that MPTP induced PD in "frozen" addicts (101–103). Mitochondrion is the major source of cellular energy. Each cell has thousands of mitochondria throughout the cytoplasm. In addition to generating most of the energy in the form of adenosine triphosphate (ATP) required by the cell through the oxidative phosphorylation system (OXPHOS), mitochondria also generate and remove free radicals and play the central role in initiating many of the key steps for apoptosis (104). Mitochondrial dysfunctions are now recognized to be the major cause of nigral degeneration in experimental models of PD (105,106) and possibly even in idiopathic PD.

Electron Transfer Chain Dysfunction

The electron transfer chain (ETC) is an important component of the OXPHOS system. The respiratory complexes I, II, III, IV, and V are located within the inner membrane of the mitochondria and play a critical role in transferring electrons from different sources within the mitochondria. Dysfunction of these respiratory complexes will lead to significant loss in the generation of stored energy in the form of ATP as well as increased oxidative damage due to accumulation of reactive oxygen species.

The MPTP model of PD clearly suggests that inhibition of the ETC, especially at the Complex I level, is toxic to nigral neurons (107). There is a

significant decrease in the levels of complex I in the nigral neurons of PD (108,109). Among the different diseases of the basal ganglia, the deficiency of Complex I in the nigra may be specific to PD (110). Complex I deficiency is not restricted to the brain, but is also found in the skeletal muscle, platelets, fibroblasts, and lymphocytes (111) in PD. It is important to recognize that in the two most commonly used animal models of PD, 6-OHDA– and MPTP-induced models, the toxins decrease the efficiency of Complex I (105,106).

Chronic injections of a commonly used pesticide, rotenone, cause selective degeneration of the nigrostriatal system and result in aggregation of ubiquitin and α-synuclein (112). This is important in understanding the etiology of PD. Rotenone, a toxin that is used to kill fish in ponds, is a mitochondrial toxin, which easily crosses the blood-brain barrier and inhibits Complex I. The clinical syndrome that results with chronic rotenone administration has significant similarities to human PD, including hypokinesia, stooped posture, and tremor. A dysfunction of Complex III has also been suggested to occur in PD (113). Recent studies suggest that nitric oxide (NO) can inhibit Complex IV, and this inhibition may accentuate the toxic effects of methyl-4-phenylpyridium(MPP+) on Complex I (114).

Free Radicals and Mitochondrial Toxicity

Mitochondria are the major source of energy production for the cell and the major site of utilization of cellular oxygen. During the synthesis of stored energy in the form of ATP, mitochondria produce several reactive oxygen species (ROS). It is estimated that 2–4% of the utilized oxygen is converted into ROS. ROS consists of superoxide anions, hydroxyl radicals, and hydrogen peroxide. The majority of the ROS produced in a cell is derived from the mitochondria. ROS are produced at Complex I and Complex III of the ETC system (115).

ROS are toxic to neurons. An increased level of ROS within the mitochondria can decrease the efficiency of Complex I and the ETC system. This will decrease energy production and cause further accumulation of free radicals, mtDNA damage, and production of additional free radicals through Fenton reaction. All of these events may induce aging of the cell and mitochondria-mediated apoptotic mechanisms of cell death (115). An increased accumulation of ROS can occur either due to increased synthesis of ROS or because of failure or a decreased level of removal by the glutathione system. Evidence for both increased production as well as decreased clearance of these free radicals has been observed in PD.

The different metabolic breakdown pathways of levodopa may be an important source of an increased production of free radicals. There is controversy regarding the neurotoxic vs. neuroprotective role of levodopa in PD (116–118). There is extensive literature to suggest that, at least in experimental conditions, especially in conditions using cell culture techniques, levodopa can produce free radicals and can destroy the cells in culture. In the presence of iron, there is an even greater increase in the levels of free radicals synthesized (119,120). Extensive review of this issue suggests that the evidence supporting the neurotoxic role of levodopa is insufficient in patients with PD (118). In fact, the cytotoxic effects of dopamine may be an artifact of cell culture technique (121), and the neurotoxic effects of accumulated iron may be due to its ability to promote aggregation of α-synuclein (84) and may contribute to Lewy body pathology.

An increased level of free radicals may be from sources other than the mitochondria. NO may be one such source of free radicals (122). An increased production of nitric oxide synthase, as noted in MPTP models of PD (123), can lead to increased levels of NO, which in turn results in an increased synthesis and accumulation of free radicals within the nigral cells. NO may play an important role in inducing nigral degeneration in amphetamine and MPTP-induced PD. Increased NO levels can lead to increased formation of peroxynitrite, a potent oxidant, and peroxynitrite may interact with dopamine autooxidant products and neuromelanin (124,125) and can cause free radical mediated nigral neurotoxicity.

The presence of a defective free radical removal system has been established in PD. ROS is removed by superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD facilitates the conversion of superoxide to hydrogen peroxide, and catalase and glutathione peroxidase convert hydrogen peroxide to water (115). Decreased levels of glutathione, glutathione peroxidase, and catalase have been observed in PD (126,127). In fact, glutathione depletion may be one of the earliest events in the evolution of mitochondrial dysfunctions in PD (128). Decreased glutathione may actually cause a selective inhibition of Complex I and result in an inefficient ETC system (129,130).

mtDNA Defects

Mitochondria's own genome (mtDNA) is localized in the matrix compartment. mtDNA consists of a 16.5 kb molecule coding for a total of 37 genes, 13 of which code for the oxidative phosphorylation system (OXPHOS), 2 for ribosomal RNAs (12S and 16S rRNA), and 22 for transfer RNAs (tRNA), molecules that are necessary for the translation of mtDNA

structural genes (104,131). Mutations of mtDNA increase with age. Cybrid studies have suggested that the Complex I deficiency noted in idiopathic PD may be due to mtDNA aberrations. Transmitochondridal cybrid lines, using mitochondria of platelets from PD patients, show several features of oxidative stress, increased vulnerability to MPP+, and an increased expression of apoptotic molecules (132,133). More recently, the presence of tRNA mutations have been observed in histologically confirmed PD patients (134).

Summary

This evidence suggests that toxins in the environment and toxins generated intrinsically can result in dysfunction of nigral mitochondria and lead to nigral degeneration and the induction of a slowly progressive syndrome similar to PD.

NIGRA AND APOPTOSIS

There is a significant controversy about whether apoptotic mechanisms play a role in the death of substantia nigra neurons in PD (135,136). However, experimental evidence of several well-recognized markers of apoptosis has been demonstrated in the nigral cells of both experimental models of PD and human PD (137).

Apoptosis, an established mechanism of cell death during embryogenesis and brain maturation, is characterized by a well-defined stereotypical pattern of neurochemical and morphological changes (138,139). The process involves caspase-induced cleaving of DNA and numerous other intracellular polypeptides, which ultimately leads to cellular death. Activation of caspases is a key triggering event for apoptosis. Caspases may be activated by at least by two different mechanisms: an extrinsic system which involves stimulation of death receptors by extracellular "death ligands" (139) or an intrinsic pathway, which requires the activation of the mitochondrial pathway. Death ligands bind to members of TNF receptor gene superfamily of death receptors, namely Fas, TNFR1, DR3, DR4, and DR5 (38), and cause apoptosis. Once stimulated, the death receptors activate the initiator caspase 9, and this in turn activates effector caspases 3 and 6 and executes apoptosis.

Mitochondrial Apoptotic Pathways and the Substantia Nigra

Many apoptotic and antiapoptotic factors converge upon the mitochondria. The expression of many of these factors is dependant on the activation of

neurotrophin and *trk*-mediated antiapoptotic or the $p75^{NTR}$-mediated apoptotic molecular cascades (Fig. 5). The members of the Bcl-2 family, namely Bcl-2, Bcl-xl, and Bcl-w, are antiapoptotic. Bax, Bak, and another group of polypeptides that includes Bid, Bad, Bim, and Bik are proapoptotic (139) (Figs. 4 and 5). Activation of caspases through the mitochondrial pathway is mediated by proapoptotic molecules facilitating the release of cytochrome *c* from the mitochondria as well as apoptotic-inducing factor (AIF) and endonuclease G, two other molecules released from the mitochondria that participate in apoptosis. Release of cytochrome *c* into the cytoplasm triggers activation of initiator caspase 9, formation of apoptosome, and subsequent activation of the effector caspases 3 and 6 and results in the destruction of the cytoplasm. The AIF translocates to the nucleus and contributes to DNA fragmentation and chromatin destruction and ultimately to cell death (139).

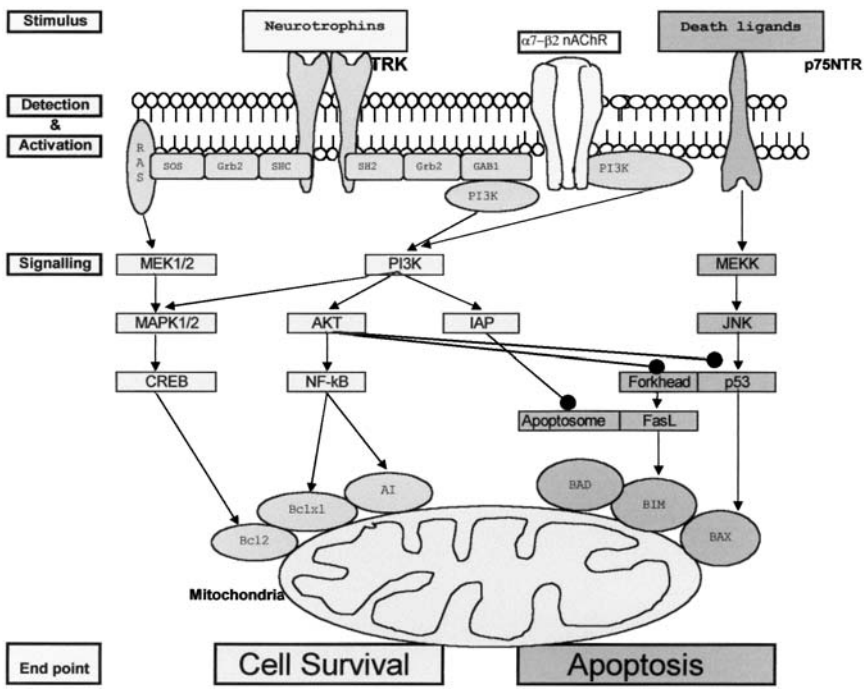

FIGURE 5 The neurotrophin-mediated pathways influencing the expression of different proapoptotic and antiapoptotic molecules on the mitochondria. (Adapted from Refs. 35,139,158.)

Bax, the proapoptotic protein, is expressed ubiquitously in normal and degenerating neurons of human nigra, but the numbers of Bax-positive neurons are significantly higher among the melanized degenerating nigral neurons in PD (140,141). p53-deficient mice were resistant to MPP+-induced neurotoxicity of dopaminergic neurons of nigra (142). Bax ablation prevents the MPTP-induced mouse model of PD (143). Overexpression of Bcl-2 protects catecholaminergic neurons from MPP+ and 6-OHDA toxicity of PC12 cells and neurons (144,145).

The effector caspase, caspase 3, is activated in 6-OHDA models of PD (146–149). Similarly, MPTP-induced nigral neurotoxicity has also been shown to be apoptotic. MPP+ also induces significant elevations of levels of caspase 3, caspase 8, and caspase 1 in the nigral neurons of mice, and inhibition of caspase activity prevents MPP+ neurotoxicity (150). Caspase 1 and 3 activities are increased in human nigral dopaminergic neurons of PD (151–153).

TUNEL-positive cells, an indicator of DNA fragmentation from apoptosis, have been noted in the MPTP-induced degeneration of nigral neurons and in melanized dopaminergic neurons of human nigra in PD (154–156). These and other studies strongly suggest that activation of the molecular cascades of proapoptotic pathways plays an important role in the death of substantia nigra neurons in PD.

Death Receptors and the Substantia Nigra

Apoptosis can also be induced by activation of several receptors of the TNF receptor gene superfamily. When "death ligands" bind to the receptors, the cells trigger several molecules that instruct the cells to self-destruct. So far, the ligands that directly activate the death receptors in PD have not been identified. TNF-receptor1, a receptor that contains the "death domain," is expressed in the nigral dopaminergic neurons and the glial cells that express this receptor. These are higher in the substantia nigra of PD than in controls (157).

Summary

The evidence for mitochondrial apoptotic pathways playing an important role in the death of dopaminergic neurons in PD is being established. The possible role of the receptors with death domain in the pathogenesis of PD and the role of increased levels of cytokines that have the potential to stimulate these death receptors is just beginning to be explored.

CONCLUSION

From the evidence discussed above, it is clear that both environmental and genetic factors can cause a parkinsonian syndrome that is similar to idiopathic PD. Environmental toxins induced mitochondrial dysfunction might be the most common cause of PD. In this regard, the observation that chronic administration of rotenone, a common toxin, can lead to a neurochemical and clinical syndrome that has significant similarities to that of idiopathic PD is a seminal one. This study certainly reinforces the prevailing epidemiological evidence that environmental toxins may cause PD.

However, based on the pathogenesis of familial PD, it is also recognized that dysfunction of the ubiquitin-proteasome system can lead to protein aggregation and resultant toxicity to nigral neurons. Accumulation and aggregation of proteins because of abnormal folding of proteins or inefficiency of molecular chaperones or proteasomal functions may occur even in the absence of any gene defects or toxins. Such mechanisms may play a role in aggregation of proteins in non-familial forms of Alzheimer's, prion and motor neuron diseases (68,75,78).

The survival of a neuron may also be dependant on a delicate balance between the neurotrophin and *trk* receptor–mediated antiapoptotic pathway and the proapoptotic pathway mediated by $p75^{NTR}$ and other death receptors (139,158). As of yet, there is no direct evidence to support the hypothesis that withdrawal of growth factors or stimulation of the death receptors and accessing the direct pathway to apoptosis exists in PD. However, the role of a decreased level of growth factors as well as increased levels of cytokines observed in the dopaminergic neurons of the substantia nigra in PD remains to be explored.

As the understanding of the trophic factors that influence normal differentiation and maturation of nigral dopaminergic neurons is expanding, the possibility that lack of or reduced influence of these ontogenic trophic factors may somehow result in either a decreased number or defective dopamine neurons in adulthood exists. Such a decrease in the number or efficiency of dopamine neurons may be a risk factor for developing PD later in life.

While many of these concepts about multiple etiologies for PD are nothing more than hypothetical at present, the knowledge derived from modern experimental approaches will certainly allow us to enter into a new and exciting phase of diagnosing these patients early in the course of the disease and treat them with molecules that will either slow the progression of PD or, hopefully, even stop the progression of the disease.

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