Etiology of Parkinson's Disease

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ABSTRACT: Controversy over the etiology and pathogenesis of Parkinson's disease (PD) has continued for many years and while the details have changed, the uncertainty persists. Although heritability was most emphatically refuted a decade ago by many investigators, recent progress firmly indicates that genetic factors at least play a role, although probably to a variable degree from one individual to another. Evidence for a variety of other etiological factors is amassed from epidemiological studies, animal models, molecular and cellular biology. Genetic factors, infectious and immunological abnormalities, the effects of ageing, toxins (endogenous as well as exogenous) and other environmental factors may all contribute to the development of PD. Loss of nigral dopaminergic neurons may be mediated by varying combinations of oxidative free radical toxicity, impaired mitochondrial function, "weak excitotoxicity" and abnormal handling of cytoskeletal proteins, all of which may shift the balance regulating apoptotic cell death.

R SUM : tiologie de la maladie de Parkinson. La controverse entourant l'étiologie et la pathogenèse de la maladie de Parkinson (MP) dure depuis plusieurs années et, bien que les détails ont changé, l'incertitude persiste. Bien que l'héritabilité de la maladie ait été réfutée avec emphase par plusieurs investigateurs il y a une dizaine d'années, des progrès récents indiquent clairement que des facteurs génétiques sont en cause, probablement à des degrés variables d'un individu à l'autre. Des données sur une variété d'autres facteurs étiologiques, provenant d'études épidémiologiques, de modèles animaux, de la biologie moléculaire et cellulaire, s'accumulent. Les facteurs génétiques, anomalies infectieuses et immunologiques, les effets du vieillissement, les toxines (endogènes et exogènes) et les autres facteurs environnementaux peuvent contribuer au développement de la MP. La perte de neurones dopaminergiques dans la substance noire peut être médiée par différentes combinaisons d'effets toxiques oxydatifs dus à des radicaux libres, une fonction mitochondriale altérée, une "faible excitotoxicité" et un métabolisme anormal des protéines du cytosquelette, qui peuvent tous déséquilibrer la régulation de la mort cellulaire par apoptose.

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Epidemiological Factors – General Considerations

The overall *incidence* of Parkinson's disease, based on multiple studies world-wide, is approximately 10-20 per 100,000 per annum. Estimates of PD *prevalence* vary considerably, but the currently accepted figure is approximately 200-300 per 100,000 population.[1] This is age-dependent, with prevalence rates per 100,000 ranging from approximately 5 for age under 40 to 300-700 in the 7th decade and >700 for age over 70.

Considerations of incidence, prevalence and possible causative factors should take into account the fact that diagnostic accuracy based on initial assessment is probably only about 65% and even when followed over an extended time, this improves to 76%.[2] Furthermore, many case-control studies of environmental exposure have included data derived from informants other than the patient. Inclusion of such proxy-derived data may substantially degrade the reliability of the information.[3]

Genetic Factors

In general, twin studies have shown a very low rate of concordance,[4] and this has traditionally been accepted as evidence against a major genetic basis. However, when co-twins were personally examined by the investigators, a higher than expected (3/9 monozygotic; 3/12 dizygotic twins affected) level of concordance was demonstrated.[5] Twin studies are not conclusive, even in these ideal circumstances, as subclinical abnormalities of the nigrostriatal dopamine system may not be expressed for several years. Thus, a PET study found abnormal 6-fluorodopa uptake in 40% of asymptomatic monozygotic twins of PD patients and in 28% of dizygotic twins. However, 3 of the 4 "asymptomatic" MZ twins had tremor at the time of the PET scan (and 1 went on to develop PD within 2 years).[6] The largest twin study to date, based on 19,842 white male twins enrolled in the National Academy of Sciences/National Research Council World War II Veteran Twins Registry was recently reported. Concordance rates were similar in monozygotic and dizygotic twin pairs; however, the concordance risk was much higher when

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disease onset prior to the age of 50, suggesting that genetic factors may play a greater role in disease of earlier onset.[6a]

There is an increased incidence of family history of Parkinson's in affected individuals (16% vs. 4% in controls; age-adjusted odds ratio 3.5),[7] which may not entirely reflect genetic factors. Families with a dominantly inherited condition which is pathologically indistinguishable from PD and clinically similar have been identified, but most investigators consider these to be special cases. This includes the recently described association of familial parkinsonism with a mutation (Ala53Thr) of the gene encoding α-synuclein in the Contursi kindred, where the age of onset is younger than average, tremor is less frequent and progression is more rapid.[8,9] Although this mutation was also found in other families, they were all of Greek or Sicilian origin, raising the possibility of a founder effect. A different (Ala30Pro) mutation in this gene has been described in a German family with parkinsonism.[10] The α-synuclein mutation has now been excluded in numerous other families with dominantly inherited parkinsonism,[11,12] as well as pathologically verified sporadic PD.[13] Linkage in one family has recently been demonstrated to the short arm of the 2nd chromosome.[14] Despite the recent interest in mitochondrial abnormalities (see below), there has been no convincing evidence of maternal transmission or mitochondrial DNA abnormalities in PD, although analysis of cytoplasmic hybrids (cybrids) suggests that the abnormality of Complex I is indeed derived from mitochondrial DNA.[14a] While juvenile parkinsonism is linked to a region of the long arm of the 6th chromosome, where mitochondrial (Mn) superoxide dismutase (SOD2) is encoded, SOD2 expression was *high* in a single patient with this disorder[15] and the mutation has recently been identified in a gene called *parkin*. The N-terminal sequence of Parkin is similar to the ubiquitin family of proteins and it is therefore possible that juvenile parkinsonism results from impaired proteolytic processing.[16] The importance of this mechanism is further supported by the recent identification of an Ile93Met mutation of the ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) gene in a German pedigree with typical, adult-onset parkinsonism.[16a] Genetically inherited susceptibility to certain toxins has been suggested by diminished debrisoquine hydroxylation, sulfoxidation and *S*-methylation reactions. An increased incidence of mutant alleles of the *CYP2D6* gene has been reported by multiple groups, but this does not seem to segregate with disease expression within families with multiple affected members. Similarly, reports that PD may be associated with increased frequency of an MAO-B allele[17] have not been consistently reproduced. A number of other candidate genes (*GPX1, TH, BDNF, CAT, APP* and *SOD1*, as well as *CYP2D6*) have been excluded in at least some families.[18,19]

Nature *vs.* Nurture – Population Studies

Although PD is in general widespread, some populations seem to have a lower incidence. This would include South African and Nigerian blacks, although blacks living in Mississippi are affected to a comparable degree as the white population[20] and a recent autopsy study suggests that black Africans have an equivalent prevalence of incidental Lewy body disease.[21] Lower incidences have likewise been reported in Oriental populations (but not in Oriental Americans and possibly not in Taiwanese).[22,23] While many investigators have reported an increased risk of having a close relative with PD in patients compared to controls, one interesting study of families in which members of multiple generations were affected suggested that disease onset clustered around the same *calendar* year rather than a comparable *chronological* age,[24] again suggesting shared exposure (and susceptibility?) to some unidentified environmental factor.

Epidemiological studies have also led to more direct support for an environmental hypothesis. Young-onset parkinsonism has been associated in a number of studies with exposure to well water.[25] No specific toxin has been identified and the well water link has not been found in all studies, although a more consistent factor seems to be rural upbringing. One compelling rural factor is pesticide/herbicide exposure, although this has also not been reproduced in all studies.[26-29] The association may be strengthened by a recent post-mortem report of increased detection of the lipid-soluble pesticide (and mitochondrial toxin) dieldrin in the brains of Parkinson patients compared to Alzheimer and normal controls.[30]

While rural environments are associated with an increased risk of PD in the *industrialized* world, the opposite seems to be true in China, where exposure to industrial chemicals is less likely to occur in the countryside. In China, consumption of well water or high ethanol intake are associated with a *reduced* risk of Parkinson's, whereas the risk is *increased* in those who consume river water or live in proximity to rubber plants.[31] Data from the Canadian study on ageing recently suggested a relationship to plastic or epoxy resin exposure.[32]

Numerous investigators have suggested an inverse relationship between smoking and Parkinson's disease, but this finding has remained controversial and most of the reports were based on case-control studies.[33-36] Whereas a recent case-control study confirmed a lower prevalence of *current* smoking in Parkinsonians, but no difference in prior exposure (suggesting that there is no protective effect, but rather that PD itself leads to reduced smoking),[34] another *prospective* study of more than 8,000 men enrolled in the Honolulu Heart Program did indeed support a reduced risk of Parkinson's in smokers or ex-smokers (relative risk = 0.39), with an apparent dose-response effect.[37] If the relationship is indeed a real one, there still exists the question of whether it reflects a "rigid" premorbid personality trait, as has been repeatedly described in PD, or a lower propensity to nicotine addiction, rather than a "protective" effect of smoking, perhaps mediated by stimulation of toxin-neutralizing enzymatic pathways.

Some retrospective studies have found an increased risk of PD following head injury,[27-29] but this has been quite variable.[38]

Infectious and Immunologic Mechanisms

The pandemic of encephalitis lethargica in the early 20th century led to parkinsonism which was clinically and pathologically distinct from idiopathic disease, but there were nonetheless expectations that an infectious etiology might be found for the "idiopathic" disorder. Sporadic post-encephalitic parkinsonism does still occur, albeit very rarely, but repeated efforts to identify an infectious agent which is consistently associated with the development of PD have failed. Interest has been rekindled by the appearance of clinically typical parkinsonism in patients with encephalitis, with image evidence of damage confined to the

substantia nigra,[39] as well as the demonstration of relatively selective involvement of the substantia nigra by neurovirulent influenza A.[40] Parkinsonism is not transmissible to primates, effectively ruling out a "slow viral" or prion-related etiology.

Activated microglia are seen in the substantia nigra of patients dying with Parkinson's disease.[41] Whether or not these reflect a response to ongoing cell death as opposed to the primary mechanism of neuronal degeneration is, however, unresolved. Although there have been reports of disease-specific antineuronal antibodies in the CSF and of complement-dependent dopaminergic toxicity in PD serum,[42] there has been no direct evidence to suggest a primary immunological abnormality in PD.

Is Parkinson's a Response to Normal Ageing?

Normal ageing is associated with clinical features which are somewhat reminiscent of PD, including the assumption of a stooped posture, slowing of body movements and a reduction of associated movements. Ageing is associated with a linear decline of pigmented neurons in the substantia nigra[43] and with decreased levels of striatal dopamine, tyrosine hydroxylase and dopa decarboxylase. This has led to the suggestion that PD may result from the effects of ageing superimposed upon an insult to the nigrostriatal system earlier in life, which may go unrecognized, as symptoms of parkinsonism do not become apparent until dopamine depletion exceeds 50%. However, the pattern of age-related striatal dopamine depletion is different from PD[44] and the presence of activated microglia at all stages of PD[41] suggests that there is an ongoing active process. The original ageing hypothesis has been revised, based on an analysis of rates of disease progression estimated from repeated clinical examination.[45] It is now proposed that the rate of PD progression is compatible with either an event which kills some nigral neurons and damages others, leading to reduced survival, or with an event which initiates a process that subsequently unfolds at a constant rate.[46,47]

Toxins and Parkinson's Disease

A variety of toxins have been associated with the development of parkinsonism and neuronal death, including cyanide, manganese, carbon disulfide, methanol and organic solvents. In general, however, the pattern of neuronal death is different from that seen in idiopathic PD and clinical features are variable. As noted above, PD may be associated with exposure to well water, pesticides, epoxy resins or other chemicals, but to date, no specific toxic constituent has been identified. Thus, the identification in the early 1980s of a group of drug addicts who developed typical clinical features following exposure to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and the concurrent demonstration that MPTP is a selective nigral toxin in primates was a significant breakthrough. In non-human primates, MPTP leads to selective depletion of dopamine and preferential destruction of the A9 dopamine neurons, but lesser involvement of the A10 neurons and locus coeruleus are also seen. Furthermore, aged animals exposed to MPTP may develop structures resembling Lewy bodies. Some strains of mice develop dopamine depletion, but nigral cell death is not universally observed,[48,49] with strain-related differences. Rats are resistant to peripherally administered MPTP, in part related to catabolism in capillary walls,[50] but also reflecting reduced sensitivity to MPP+,[51] although aged rats are partially susceptible and MPP+ is partially effective following direct intracerebral administration.[52,53]

MPTP undergoes MAO-B-dependent conversion to MPP+ in glial cells. MPP+ is then in turn released into the extracellular space and selectively taken up by DA neuronal terminals (dependent upon the membrane DA transporter), where it then inhibits mitochondrial Complex I activity.[54] A variety of naturally occurring isoquinolines which are structurally analogous to MPTP, inhibit mitochrondrial Complex I[55] and can enhance apoptotic death in dopaminergic neurons[56] have been implicated as endogenous neurotoxins in PD.

There is a considerable amount of controversial literature on the relationship between MPTP toxicity and excitatory amino acid transmission. Excitatory amino acid inhibitors might be expected to reduce the *symptoms and signs* of PD by blocking excessive output from the subthalamic nucleus, but the potential role of excitatory amino acids as *endogenous toxins* in PD is less clear. Some investigators have reported that MPTP or MPP+ toxicity can be prevented by the non-competitive NMDA antagonist MK-801[57] and MPP+ enhances glutamate toxicity to dopaminergic neurons in culture.[58] It has been suggested that the acute phase of MPTP/MPP+ toxicity may be associated with excessive depolarization-induced stimulation of glutamate receptors and that neurons are more susceptible to the resultant influx of calcium ions because of impaired mitochondrial function (the "weak excitotoxic" hypothesis).[59] There is indeed an excitatory amino acid input to the substantia nigra from the pedunculopontine and subthalamic nuclei, but many labs have failed to reproduce the protective effects of MK-801 in this model and the proposed mechanism remains speculative. However, protection against 6-hydroxydopamine-induced nigral degeneration following subthalamic nucleus lesions in the rat has also been described.[60] Given that amantadine has properties as an excitatory amino acid antagonist, a novel rationale for this medication in Parkinson's disease is suggested and indeed its use has been associated with prolonged survival.[61]

Endogenous Toxins – the Free Radical Hypothesis

A converging amount of evidence suggests that Parkinson patients may suffer the combined effects of multiple factors culminating in free radical-induced damage. MAO-catalyzed oxidative deamination of dopamine results in the formation of hydrogen peroxide. Dopamine additionally undergoes autoxidation reactions which result in the formation of reactive quinones and semiquinones (also the mechanism of 6-hydroxydopamine toxicity) as well as the superoxide anion radical, the latter subsequently cleared by superoxide dismutase to form more hydrogen peroxide. Under usual conditions, hydrogen peroxide is cleared by reduced glutathione (via glutathione peroxidase) or catalase, but in Parkinson patients, there is a deficiency of reduced glutathione (GSH) which appears to be relatively disease specific and restricted to the substantia nigra.[62] A partial deficiency is also seen in the brains of people with incidental Lewy body disease.[63] This deficiency may in part be a *consequence* of increased oxidative stress (via conversion to oxidized glutathione), but may also reflect *degradation* secondary to increased activity of γ-glutamyltranspeptidase.[64] This depletion of GSH may lead to impaired clearance of hydrogen peroxide, which is now available

to undergo non-enzymatic (Fenton) reactions with ferrous ions, resulting in the formation of the highly toxic hydroxyl radical. The resultant ferric ions can also react with the superoxide anion radical to form hydroxyl radicals (Haber-Weiss reaction).[65,66] Hydroxyl radicals are highly reactive and will in particular result in the formation of lipid peroxides which damage cell membranes.

Interest in oxyradical mechanisms of neurodegenerative disease increased with the identification of *SOD1* mutations in some cases of familial ALS.[67] However, motor neuron degeneration is associated with a *gain* of mutant *SOD1* function[68] and degeneration may in part be mediated by peroxynitrite formation;[69] *SOD* mutations have not been associated with idiopathic PD. In juvenile autosomal recessive parkinsonism secondary to mutations of the *parkin* gene on chromosome 6q, there may be a gain of *SOD2* function.[15]

What evidence, direct or indirect, suggests that this cascade of events might occur in PD? Nigral iron content is increased in PD substantia nigra and it may furthermore be insufficiently "trapped" by ferritin, which is (variably) deficient.[69a,b] Neuromelanin can bind iron and facilitate Fenton and Haber-Weiss reactions. However, 6-hydroxydopamine-induced degeneration of the substantia nigra also results in elevated iron levels, as do multiple other degenerative conditions, so this finding may be secondary. Deficiency of reduced glutathione has been demonstrated, as have changes in SOD, which are less consistent, but would nonetheless be compatible with oxidant stress. Depletion of GSH alone fails to result in nigral cell loss,[70] but potentiates MPTP or MPP+ toxicity.[71] In cortical neurons, GSH deficiency results in cell death mediated by *12-lipoxygenase*.[72] A number of studies have suggested abnormal lipid peroxidation in PD[73] and further evidence of free radical toxicity is derived from the demonstration of increased 8-hydroxy-2'-deoxyguanosine, a marker of oxyradical-mediated DNA damage, in PD brain.[74] This increase was seen in all brain regions studied (except the cerebellum), however, and may be secondary to L-DOPA therapy, so the pathogenetic significance of this finding is as yet unresolved.

Mitochondrial Dysfunction

A number of studies now suggest that there is an abnormality of nigral mitochondrial Complex I in PD. This appears to be relatively region specific within brain, but abnormalities in other tissues (platelets and muscle) and of other components of the electron transport chain have been less consistent.[75,76] Some degree of disease specificity has also been demonstrated, suggesting that this does not simply reflect cell loss. It is of interest that MPP+ also exerts its toxicity by inhibition of Complex I, suggesting a close link to idiopathic PD. Complex I abnormalities may lead to increased formation of oxyradicals which are normally tightly bound to the electron transport chain. Indeed, transgenic mice with high Cu/Zn (cytosolic)-SOD activity are resistant to the toxic effects of MPTP and MPP+.[77] Mitochondrial abnormalities may also result in impaired formation of reduced glutathione. Conversely, oxyradicals are toxic to mitochondria and the Complex I abnormalities may be secondary. The brains of people with incidental Lewy body disease demonstrate a deficieny of reduced glutathione which is intermediate between normal controls and Parkinson subjects, but the reduction of Complex I activity was not statistically significant.[63] Furthermore, systemic administration of the specific complex I inhibitor rotenone induced striatal and pallidal, but *not* nigral cell loss.[78] Although 7 of the approximately 40 subunits of Complex I are encoded by mitochondrial DNA, there has been no convincing evidence of altered mitochondrial DNA in PD.

The indirect evidence to support the free radical/mitochondrial hypothesis is compelling, but caution is warranted before it is accepted as the primary abnormality as opposed to either a series of secondary abnormalities or a contributor to ongoing cell death in the presence of another as yet unidentified process. These issues have been reviewed in detail in a number of excellent reviews and critiques.[79-81]

Implications of the Free Radical Hypothesis

If the events outlined above are indeed important for the pathogenesis of PD, then it is potentially possible to intervene in the disease process. MAO-B inhibitors might theoretically help by preventing enzymatic oxidation of dopamine, while other antioxidants (Vitamins C and E) may quench free radicals. To date, there is no convincing evidence that any of these compounds affect disease progression,[82] although Vitamin E deficiency may lead to (asymptomatic) impairment of nigrostriatal function.[83] Although abnormalities of iron are likely to be largely secondary, chelation might be of theoretical interest to slow down further disease progression. While protective effects have been demonstrated in animal models, this approach has not been tested in humans.

One of the most disturbing issues is the question of whether levodopa itself may contribute to ongoing nigral cell death in PD, by increasing dopamine turnover and promoting oxyradical formation. While high concentrations of levodopa and dopamine are indeed toxic in cell culture systems (in the absence of glial cells; levodopa actually has *trophic* effects in neuronal-astrocyte cocultures)[84] and may also reduce nigral graft survival in animal transplant models, there has been no convincing evidence that levodopa hastens disease progression in PD.[85] If anything, levodopa use has been associated with *enhanced* survival of remaining dopamine neurons in rats with partial 6-hydroxydopamine lesions[86] and with decreased mortality in PD,[87] which of course probably reflects symptomatic benefit. Nonetheless, the issue remains controversial and the oxyradical hypothesis has led some authors to suggest that symptomatic therapy should be initiated with dopamine agonists (which will actually reduce dopamine turnover) rather than levodopa. Dopamine agonists may lead to decreased free radical production, seem to retard the normal age-related loss of nigral dopamine neurons in rodents and their use may be associated with decreased mortality in PD.[88] Whether or not their use as *de novo* therapy confers any advantage over initiating treatment with levodopa is the subject of a number of ongoing clinical trials.

Trophic Factors

Target-derived trophic factors are substances which are produced and released in limited quantities in the projection areas of neurons which respond to them. Within the nervous system, the primary effect is on survival and differentiation of neurons during embryonic development, as well the maintenance of neuron-specific function during adult life. Recent advances in molecular

biology have led to an explosion of interest in this area, with the identification of novel factors and better understanding of receptor structure and transduction mechanisms. These compounds may prove to be beneficial in a variety of neurodegenerative disorders, including Parkinson's disease, and may also substantially promote the survival and function of neuronal grafts used for the treatment of these conditions.

The trophic factors attracting the greatest attention in PD are brain derived neurotrophic factor (BDNF), a member of the neurotrophin (nerve growth factor-related) family, which interacts with the *trk*B receptor, and glial cell-derived neurotrophic factor (GDNF), which belongs to the TGF (transforming growth factor)-β superfamily. BDNF promotes the survival of mesencephalic dopaminergic neurons in culture and protects against MPP+ toxicity.[89] Although initially thought to be highly selective for dopaminergic neurons,[90] GDNF is now recognized to have trophic influences on other neuronal populations, particularly motor neurons. GDNF selectively promotes dopamine uptake, dopamine neuronal survival and morphological differentiation in rostral mesencephalic tegmental cultures. GDNF is expressed in dopamine target areas during development and is transported retrogradely in the nigrostriatal pathway.[91] Although its message is not upregulated by 6-hydroxydopamine lesions, GDNF markedly enhances survival of fetal dopaminergic grafts and recent studies have shown beneficial effects in MPTP, 6-hydroxydopamine and axotomy models of parkinsonism.[92,93] Recent animal studies indicate that GDNF and other neurotrophic factors can be expressed in microencapsulated cells[94] or by modified viral vectors,[95] thus offering a novel approach to either enhance the survival of mesencephalic grafts used for the treatment of PD, or possibly even to delay disease progression.

Cyclosporin and the immunosuppressant FK506 both bind to proteins which interact with calcineurin. The recently described FK506 analogue GPI 1046 has been shown to have neuroprotective effects in 6-hydroxydopamine and MPTP-induced parkinsonism in rodents.[96] This effect seems to be independent of action on calcineurin and GPI 1046 has no immunosuppressant properties. Other proteins of interest, which seem to preferentially promote the survival and differentiation of dopaminergic nigral neurons include sonic hedgehog,[97] the GDNF analogue neurturin,[98] and the orphan nuclear receptor family member Nurr-1.[99]

While all these factors may be of interest in treating PD, there is no evidence to date to suggest that PD is associated with a specific deficiency of trophic factors, with the possible exception of basic fibroblast growth factor (bFGF), whose expression is reduced in nigral neurons of PD patients.[100] Many of the neurotrophic factors are known to be expressed by glial cells. Thus, future studies on the mechanisms underlying neurodegeneration may need to pay closer attention to the possibility of abnormal glial function. This also highlights importance of providing an adequate glial support base when studying the effects of various manipulations on dopaminergic neurons *in vitro* (e.g., as noted above, levodopa is toxic to dopamine cell cultures in isolation, but has a trophic effect when studied in mixed neuronal-glial cultures).[84]

Apoptosis

While a variety of insults may lead to cell necrosis, often accompanied by an inflammatory response, *normal* cells may undergo death followed by rapid phagocytosis, particularly if deprived of trophic factors. This programmed cell death appears to be important for the regulation of cell number, the prevention of tumours and the appropriate mix of cell types (e.g. neurons only survive in the presence of the appropriate target tissue). Programmed cell death is an active, gene-regulated process which is morphologically distinct from necrosis. In addition to the absence of an inflammatory response, characteristic features include cell shrinkage, clumping of nuclear chromatin and cytoplasmic and nuclear fragmentation (apoptosis). This phenomenon has been most extensively studied in the worm *Caenorhabditis elegans*, where the genes *ced-3* and *ced-4* promote cell death and their inactivation leads to prolonged survival. The gene *ced-9* opposes this function and a mammalian analogue, *bcl-2* has been identified, which can prevent apoptosis when expressed in *C. elegans*. The fate of mammalian cells may be determined by the balance between anti-apoptotic proteins such as *Bcl-2* or *Bcl-x* and opposing proteins such as *Bax*. The mammalian analogue of *CED-3* is interleukin-1β converting enzyme (ICE), whose death-promoting properties can be prevented by the product of the *crmA* (cowpox virus ***c***ytokine ***r***esponse ***m***odifier) gene[101] and by the baculovirus antiapoptotic protein p35.[102] ICE inhibitors arrest motoneuron death resulting from deprivation of trophic factors *in vitro*.[103] ICE itself is not usually involved in apoptosis, but the related family of caspases are, in a self-activating cascade that may be triggered by extracellular (*e.g.,* activation of the cell surface receptor Fas) or intracellular (*e.g.,* release of mitochondrial cytochrome c) factors.[104]

The role of apoptosis in Parkinson's is as yet unresolved. One report described apoptosis in the substantia nigra of 8 of 11 parkinsonian patients studied post-mortem using the TUNEL (Terminal deoxynucleotidyl transferase dUTP-biotin Nick-End Labelling) method; however, glial cells were equally affected.[105] This has been confirmed by other investigators, based on *in situ* labelling[106] or electron microscopy.[107] Other investigators have suggested that TUNEL labelling is seen in glia, but not neurons, within the SN.[108] Expression of bcl-2 protein in the nigrostriatal path of parkinsonians has been demonstrated.[109] Downregulation of SOD1,[110] inhibition of mitochondrial Complex I[111] or L-DOPA can induce apoptosis in PC12 cells and physiological concentrations of dopamine induce similar changes in cultured chick sympathetic neurons.[112] MPTP induces apoptotic nigral cell death in the mouse *in vivo*[113] While high concentrations of NMDA or superoxide/peroxynitrite induce necrosis, lower concentrations induce apoptosis,[114,115] which can be differentially affected by specific trophic factors. The recent demonstration of increased nuclear translocation of NF-κB in nigral dopamine neurons of Parkinsonian patients[116] has been interpreted as evidence of apoptosis triggered by oxidative stress; however, the role of NF-κB itself (promotion of cell death vs. protection) is unclear.

Other Factors of Potential Relevance

The role of other factors which determine dopaminergic cell fate during development in the pathogenesis of Parkinson's disease is not understood. One such factor which has attracted considerable interest is *sonic hedgehog*, a protein which is expressed in notochord and floor plate cells and induces a ventral cell phenotype.[97] The *weaver* mutation is a spontaneously arising defect

in mice, characterized by degeneration of cerebellar granule and midbrain dopaminergic neurons. It has been linked to a mutation of G protein-gated inward rectifying potassium (GIRK) channels.[117] Further details on the mechanisms of nigral cell death are not yet understood, but it appears to be non-apoptotic.

SUMMARY

This seemingly confusing array of etiological possibilities does not represent a series of mutually exclusive events. It seems clear that Parkinson's is associated with excess generation of free radicals and impaired function of mitochondrial Complex I, although whether one of these may lead to the other and whether either is of primary pathogenetic importance is still unresolved. Genetic and environmental factors probably both influence these processes, to varying degrees from one individual to another. Whether a deficiency of trophic or other protective "anti-apoptotic" factors contributes to the development of Parkinson's is also unclear. Recently discovered mutations of α-synuclein and parkin suggest that increased attention should be paid to protein handling. Improved understanding of these issues will allow the development of more rational treatment strategies for Parkinson's as well as other neurodegenerative disorders.

REFERENCES

1. Rajput AH. Frequency and cause of Parkinson's disease. Can J Neurol Sci 1992; 19 (Suppl. 1): 103-107.
2. Rajput AH, Rozdilsky B, Rajput A. Accuracy of clinical diagnosis in Parkinsonism-a prospective study. Can J Neurol Sci 1991; 18: 275-278.
3. Semchuk KM, Love EJ. Effects of agricultural work and other prox-derived case-control data on Parkinson's disease risk estimates. Am J Epidemiol 1995; 141: 747-754.
4. Ward CD, Duvoisin RC, Ince SE, Nutt JD, Eldridge R, Calne DB. Parkinson's disease in 65 pairs of twins and in a set of quadruplets. Neurology 1983; 33: 815-824.
5. Vieregge P, Schiffke KA, Friedrich HJ, Muller B, Ludin HP. Parkinson's disease in twins. Neurology 1992; 42: 1453-1461.
6. Burn DJ, Mark MH, Playford ED, et al. Parkinson's disease in twins studied with 18F-dopa and positron emission tomography. Neurology 1992; 42: 1894-1900.

6a. Tanner CM, Ottman R, Goldman SM, Ellenberg J, Chan P, Mayeux R, Langston JW. Parkinson disease in twins: an etiologic study. JAMA 1999; 281: 341-346.

7. Payami H, Larsen K, Bernard S, Nutt J. Increased risk of Parkinson's disease in parents and siblings of patients. Ann Neurol 1994; 36: 659-661.
8. Golbe LI, Di Iorio G, Bonavita, Miller DC, Duvoisin RC. A large kindred with autosomal dominant Parkinson's disease. Ann Neurol 1990; 27: 276-282.
9. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the a-synuclein gene identified in families with Parkinson's disease. Science 1997; 276: 2045-2047.
10. Kruger R, Kuhn W, Muller T, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nature Genetics 1998; 18: 106-108.
11. Scott WK, Staijich JM, Yamaoka LH, et al. Genetic complexity and Parkinson's disease. Science 1997; 277: 387-388.
12. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. Genetic complexity and Parkinson's disease. Science 1997; 277: 388-389.
13. Chan P, Jiang X, Forno LS, Di Monte DA, Tanner CM, Langston JW. Absence of mutations in the coding region of the alpha-synuclein gene in pathologically proven Parkinson's disease. Neurology 1998; 50: 1136-1137.
14. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nature Genetics 1998; 18: 262-265.

14a. Swerdlow RH, Parks, JK, Miller SW et al. Origin and functional consequences of the complex I defect in Parkinson's disease. Ann. Neurol. 1996; 40: 663-671.

15. Shimoda-Matsubayashi S, Hattori T, Matsumine H, et al. Mn SOD activity and protein in a patient with chromosome 6-linked autosomal recessive parkinsonism in comparison with Parkinson's disease and control. Neurology 1997; 49: 1257-1262.
16. Kitada T, Asakawa S, Hattori N, et al. Mutations in the *parkin* gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392: 605-608.

16a. Leroy E, Boyer R, Auburger G et al. The ubiquitin pathway in Parkinson's disease. Nature 1998; 395: 451-452.

17. Kurth JH, Kurth MC, Poduslo SE, Schwankhaus JD. Association of a monoamine oxidase B allele with Parkinson's disease. Ann Neurol 1993; 33: 368-372.
18. Parboosingh JS, Rousseau M, Rogan F, et al. Absence of mutations in superoxide dismutase and catalase genes in patients with Parkinson's disease. Arch Neurol 1995; 52: 1160-1163.
19. Gasser T, Wszolek ZK, Trofatter J, et al. Genetic linkage studies in autosomal dominant parkinsonism: evaluation of seven candidate genes. Ann Neurol 1994; 36: 387-396.
20. Schoenberg BS, Anderson DW, Haerer AF. Prevalence of Parkinson's disease in the biracial population of Copiah County, Mississippi. Neurology 1985; 35: 841-845.
21. Jendroska K, Olasode BJ, Daniel SE, et al. Incidental Lewy body disease in black Africans. Lancet 1994; 344: 882-883.
22. Wang SJ, Fuh JL, Teng EL, et al. A door-to-door survey of Parkinson's disease in a Chinese population in Kinmen. Arch Neurol 1996; 53: 66-71.
23. Zhang Z-X, Roman GC. Worldwide occurrence of Parkinson's disease: an updated review. Neuroepidemiol 1993; 12: 195-208.
24. Calne S, Schoenberg B, Martin W, Uitti RJ, Spencer P, Calne DB. Familial Parkinson's disease: possible role of environmental factors. Can J Neurol Sci 1987; 14: 303-305.
25. Rajput AH, Uitti RJ, Stern W, et al. Geography, drinking water chemistry, pesticides and herbicides and the etiology of Parkinson's disease. Can J Neurol Sci 1987; 14: 414-418.
26. Butterfield PG, Valanis BG, Spencer PS, Lindeman CA, Nutt JG. Environmental antecedents of young-onset Parkinson's disease. Neurology 1993; 43: 1150-1158.
27. Hubble JP, Cao T, Hassanein RES, Neuberger JS, Koller WC. Risk factors for Parkinson's disease. Neurology 1993; 43: 1693-1697.
28. Semchuk KM, Love EJ, Lee RG. Parkinson's disease: a test of the multifactorial etiologic hypothesis. Neurology 1993; 43: 1173-1180.
29. Stern M, Dulaney E, Gruber SB, et al. The epidemiology of Parkinson's disease: a case-control study of young-onset and old-onset patients. Arch Neurol 1991; 48: 903-907.
30. Fleming L, Mann JB, Bean J, Briggle T, Sanchez-Ramos JR. Parkinson's disease and brain levels of organochlorine pesticides. Ann Neurol 1994; 36: 100-103.
31. Wang W-z, Fang X-h, Cheng X-m, Jiang D-h, Lin Z-j. A case-control study on the environmental risk factors of Parkinson's disease in Tianjin, China. Neuroepidemiology 1993; 12: 209-218.
32. Chaturvedi S, Ostbye T, Stoessl AJ, Merskey H, Hachinski V. Environmental exposures in elderly Canadians with Parkinson's disease. Can J Neurol Sci 1995; 22: 232-234.
33. Godwin-Austen RG, Leigh PN, Marmot MG, Stern GM. Smoking and Parkinson's disease. J Neurol Neurosurg Psychiatry 1982; 45: 577-581.
34. Mayeux R, Tang MX, Marder K, Cote LJ, Stern Y. Smoking and Parkinson's disease. Mov Disord 1994; 9: 207-212.
35. Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology 1995; 45: 1041-1051.
36. Riggs JE. Cigarette smoking and Parkinson disease: the illusion of a neuroprotective effect. Clin Neuropharm 1992; 15: 88-99.
37. Grandinetti A, Morens DM, Reed D, MacEachern D. Prospective study of cigarette smoking and the risk of developing idiopathic Parkinson's disease. Am J Epidemiol 1994; 139: 1129-1138.
38. Williams DB, Annegers JF, Kokmen E, O'Brien PC, Kurland LT. Brain injury and neurologic sequelae: a cohort study of dementia, parkinsonism, and amyotrophic lateral sclerosis. Neurology 1991; 41: 1554-1557.

39. Lin SK, Lu CS, Vingerhoets FJG, et al. Isolated involvement of substantia nigra in acute transient Parkinsonism: MRI and PET observations. Parkinsonism Rel Disord 1995; 1: 67-73.
40. Takahashi M, Yamada T, Nakajima S, Nakajima K, Yamamoto T, Okada H. The substantia nigra is a major target for neurovirulent influenza A virus. J Exp Med 1995; 181: 2161-2169.
41. McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease of brains. Neurology 1988; 38: 1285-1291.
42. Defazio G, Daltoso R, Benvegnu D, Minozzi MC, Cananzi AR, Leon A. Parkinsonian serum carries complement-dependent toxicity for rat mesencephalic dopaminergic neurons in culture. Brain Res 1994; 633: 206-212.
43. McGeer PL, McGeer EG, Suzuki JS. Aging and extrapyramidal function. Arch Neurol 1977; 34: 33-35.
44. Kish SJ, Shannak K, Rajput A, Deck JHN, Hornykiewicz O. Aging produces a specific pattern of striatal dopamine loss: implications for the etiology of idiopathic Parkinson's disease. J Neurochem 1992; 58: 642-648.
45. Lee CS, Schulzer M, Mak EK, et al. Clinical observations on the rate of progression of idiopathic parkinsonism. Brain 1994; 117: 501-507.
46. Calne DB. Is idiopathic parkinsonism the consequence of an event or a process? Neurology 1994; 44: 4-10.
47. Schulzer M, Lee CS, Mak EK, Vingerhoets FJG, Calne DB. A mathematical model of pathogenesis in idiopathic parkinsonism. Brain 1994; 117: 509-516.
48. Hallman H, Lange J, Olson L, Stromberg I, Jonsson G. Neurochemical and histochemical characterization of neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on brain catecholamine neurones in the mouse. J Neurochem 1985; 44: 117-127.
49. Willis GL, Donnan GA. Histochemical, biochemical and behavioural consequences of MPTP treatment in C-57 black mice. Brain Res 1987; 402: 269-274.
50. Kalaria RN, Mitchell MJ, Harik SI. Correlation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity with blood-brain barrier monoamine oxidase activity. Proc Natl Acad Sci USA 1987; 84: 3521-3525.
51. Giovanni A, Sonsalla PK, Heikkila RE. Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. 2. Central administration of 1-methyl-4-phenylpyridinium. J Pharmacol Exp Therap 1994; 270: 1008-1014.
52. Heikkila RE, Nicklas WJ, Duvoisin RC. Dopaminergic toxicity after the stereotaxic adminstration of the 1-methyl-4-phenylpyridinium ion (MPP+) to rats. Neurosci Lett 1985; 59: 135-140.
53. Bradbury AJ, Costall B, Domeny AM, et al. 1-methyl-4-phenylpyridine is neurotoxic to the nigrostriatal dopamine pathway. Nature 1986; 319: 56-57.
54. Gerlach M, Riederer P, Przuntek H, Youdim MBH. MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease. Eur J Pharmacol 1991; 208: 273-286.
55. McNaught KS, Thull U, Carrupt PA, et al. Inhibition of complex I by isoquinoline derivatives structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Biochem Pharmacol 1995; 50: 1903-1911.
56. Maruyama W, Naoi M, Kasamatsu T, et al. An endogenous dopaminergic neurotoxin, N-methyl-(R)-salsolinol, induces DNA damage in human dopaminergic neuroblastoma SH-SY5Y cells. J Neurochem 1997; 69: 322-329.
57. Turski L, Bressler K, Rettig K-J, Loschmann P-A, Wachtel H. Protection of substantia nigra from MPP+ neurotoxicity by N-methyl-D-aspartate antagonists. Nature 1991; 349: 414-418.
58. Sawada H, Shimohama S, Tamura Y, Kawamura T, Akaike A, Kimura J. Methylphenylpyridinium ion (MPP+) enhances glutamate-induced cytotoxicity against dopaminergic neurons in cultured rat mesencephalon. J Neurosci Res 1996; 43: 55-62.
59. Beal MF. Aging, energy, and oxidative stress in neurodegenerative diseases. Ann Neurol 1995; 38: 357-366.
60. Piallat B, Benazzouz A, Benabid AL. Subthalamic nucleus lesion in rats prevents dopaminergic nigral neuron degeneration after striatal 6-OHDA injection: behavioural and immunohistochemical studies. Eur J Neurosci 1996; 8: 1408-1414.
61. Uitti RJ, Rajput AH, Ahlskog JE, et al. Amantadine treatment is an independent predictor of improved survival in Parkinson's disease. Neurology 1996; 46: 1551-1556.
62. Sian J, Dexter DT, Lees AJ, et al. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann Neurol 1994; 36: 348-355.
63. Dexter DT, Sian J, Rose S, et al. Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. Ann Neurol 1994; 35: 38-44.
64. Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P, Marsden CD. Glutathione-related enzymes in brain in Parkinson's disease. Ann Neurol 1994; 36: 356-361.
65. Olanow CW, Cohen G, Perl DP, Marsden CD. Role of iron and oxidant stress in the normal and parkinsonian brain. Ann Neurol 1992; 32: S1-S145.(Abstract)
66. Youdim MBH, Ben-Shachar D, Riederer P. The possible role of iron in the etiopathology of Parkinson's disease. Mov Disord 1993; 8: 1-12.
67. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993; 362: 59-62.
68. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994; 264: 1772-1775.
69. Beckman JS, Chen J, Crow JP, Ye YZ. Reactions of nitric oxide, superoxide and peroxynitrite with superoxide dismutase in neurodegeneration. Prog Brain Res 1994; 103: 371-380.
69a. Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, Lees AJ, Jenner P, Marsden CD. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. Brain 1991; 114: 1953-1975.
69b. Mann VM, Cooper JM, Daniel SE, Srai K, Jenner P, Marsden CD, Schapira AH. Complex I, iron, and ferritin in Parkinson's disease substantia nigra. Ann. Neurol. 1994; 36: 876-881.
70. Toffa S, Kunikowska GM, Zeng BY, Jenner P, Marsden CD. Glutathione depletion in rat brain does not cause nigrostriatal pathway degeneration. J Neural Transm 1997; 104: 67-75.
71. Wullner U, Loschmann PA, Schulz JB, et al. Glutathione depletion potentiates MPTP and MPP+ toxicity in nigral dopaminergic neurons. NeuroReport 1996; 7: 921-923.
72. Li YH, Maher P, Schubert D. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 1997; 19: 453-463.
73. Dexter DT, Holley AE, Flitter WD, et al. Increased levels of lipid hydroperoxides in the parkinsonian substantia nigra: an HPLC and ESR study. Mov Disord 1994; 9: 92-97.
74. Sanchez-Ramos JR, Overvik E, Ames BN. A marker of oxyradical-mediated DNA damage (8-hydroxy-2'-deoxyguanosine) is increased in nigrostriatum of Parkinson's disease brain. Neurodegeneration 1994; 3: 197-204.
75. DiMauro S. Mitochondrial involvement in Parkinson's disease: the controversy continues. Neurology 1993; 43: 2170-2172.
76. Schapira AHV. Evidence for mitochondrial dysfunction in Parkinson's disease - a critical reappraisal. Mov Disord 1994; 9: 125-138.
77. Przedborski S, Kostic V, Jackson-Lewis V, et al. Transgenic mice with increased Cu/Zn-superoxide dismutase activity are resistant to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. J Neurosci 1992; 12: 1658-1667.
78. Ferrante RJ, Schulz JB, Kowall NW, Beall MF. Systemic administration of rotenone produces selective damage in the striatum and globus pallidus, but not in the substantia nigra. Brain Res 1997; 753: 157-162.
79. Calne DB. The free radical hypothesis in idiopathic parkinsonism: evidence against it. Ann Neurol 1992; 32: 799-803.
80. Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 1992; 32: 804-812.
81. Jenner P, Schapira AHV, Marsden CD. New insights into the cause of Parkinson's disease. Neurology 1992; 42: 2241-2250.
82. Parkinson Study Group. Impact of deprenyl and tocopherol treatment on Parkinson's disease in DATATOP subjects not requiring levodopa. Ann Neurol 1996; 39: 29-36.

83. Dexter DT, Brooks DJ, Harding AE, et al. Nigrostriatal function in vitamin E deficiency: clinical, experimental, and positron emission tomographic studies. Ann Neurol 1995; 35: 298-303.
84. Mena MA, Davila V, Sulzer D. Neurotrophic effects of L-dopa in postnatal midbrain dopamine/cortical astrocyte cocultures. J Neurochem 1997; 69: 1398-1408.
85. Agid Y. Levodopa. Is toxicity a myth? Neurology 1998; 50: 858-863.
86. Murer MG, Dziewczapolski G, Menalled LB, et al. Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. Ann Neurol 1998; 43: 561-575.
87. Rajput AH, Uitti RJ, Rajput A, Offord KP. Timely levodopa (LD) administration prolongs survival in Parkinson's disease. Parkinsonism Rel Disord 1997; 3: 159-165.
88. Przuntek H, Welzel D, Blumner E, et al. Bromocriptine lessens the incidence of mortality in L-Dopa- treated parkinsonian patients: PRADO-study discontinued. Eur J Clin Pharmacol 1992; 43: 357-363.
89. Hyman C, Hofer M, Barde YA, et al. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 1991; 350: 230-232.
90. Lin L-FH, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 1993; 260: 1130-1132.
91. Tomac A, Widenfalk J, Lin L-FH, et al. Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. Proc Natl Acad Sci USA 1995; 92: 8274-8278.
92. Tomac A, Lindquist E, Lin L-FH, et al. Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature 1995; 373: 335-339.
93. Beck KD, Valverde J, Alexi T, et al. Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. Nature 1995; 373: 339-341.
94. Lindner MD, Winn SR, Baetge EE, et al. Implantation of encapsulated catecholamine and GDNF-producing cells in rats with unilateral dopamine depletions and parkinsonian symptoms. Exp Neurol 1995; 132: 62-76.
95. Choi-Lundberg DL, Lin Q, Chang YN, et al. Dopaminergic neurons protected from degeneration by GDNF gene therapy. Science 1997; 275: 838-841.
96. Steiner JP, Hamilton GS, Ross DT, et al. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. Proc Natl Acad Sci USA 1997; 94: 2019-2024.
97. Wang MZ, Jin P, Bumcrot DA, et al. Induction of dopaminergic neuron phenotype in the midbrain by Sonic hedgehog protein. Nature Medicine 1995; 1: 1184-1188.
98. Horger BA, Nishimura MC, Armanini MP, et al. Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons. J Neurosci 1998; 18: 4929-4937.
99. Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in Nurr1-deficient mice. Science 1997; 276: 248-250.
100. Tooyama I, Kawamata T, Walker D, et al. Loss of basic fibroblast growth factor in substantia nigra neurons in Parkinson's disease. Neurology 1993; 43: 372-376.
101. Gagliardini V, Fernandez P-A, Lee RKK, et al. Prevention of vertebrate neuronal death by the *crmA* gene. Science 1994; 263: 826-828.
102. Bump NJ, Hackett M, Hugunin M, et al. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. Science 1995; 269: 1885-1888.
103. Milligan CE, Prevette D, Yaginuma H, et al. Peptide inhibitors of the ICE protease family arrest programmed cell death of motoneurons in vivo and in vitro. Neuron 1995; 15: 385-393.
104. Barinaga M. Death by dozens of cuts. Science 1998; 280: 32-34.
105. Mochizuki H, Goto K, Mori H, Mizuno Y. Histochemical detection of apoptosis in Parkinson's disease. J Neurol Sci 1996; 137: 120-123.
106. Tompkins MM, Basgall EJ, Zamrini E, Hill WD. Apoptotic-like changes in Lewy-body-associated disorders and normal aging in substantia nigral neurons. Am J Pathol 1997; 150: 119-131.
107. Anglade P, Vyas S, Javoy-Agid F, et al. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 1997; 12: 25-31.
108. Kosel S, Egensperger R, vonEitzen U, Mehraein P, Graeber MB. On the question of apoptosis in the parkinsonian substantia nigra. Acta Neuropathol 1998; 93: 105-108.
109. Mogi M, Harada M, Kondo T, et al. Bcl-2 protein is increased in the brain from parkinsonian patients. Neurosci Lett 1996; 215: 137-139.
110. Troy CM, Shelanski ML. Down-regulation of copper/zinc superoxide dismutase causes apoptotic death in PC12 neuronal cells. Proc Natl Acad Sci USA 1994; 91: 6384-6387.
111. Hartley A, Stone JM, Heron C, Cooper JM, Schapira AHV. Complex I inhibitors induce dose-dependent apoptosis in PC12 cells: relevance to Parkinson's disease. J Neurochem 1994; 63: 1987-1990.
112. Ziv I, Melamed E, Nardi N, et al. Dopamine induces apoptosis-like cell death in cultured chick sympathetic neurons - a possible novel pathogenetic mechanism in Parkinson's disease. Neurosci Lett 1994; 170: 136-140.
113. Tatton NA, Kish SJ. In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. Neuroscience 1997; 77: 1037-1048.
114. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P, Lipton SA. Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc Natl Acad Sci USA 1995; 92: 7162-7166.
115. Estevez AG, Radi R, Barbeito L, Shin JT, Thompson JA, Beckman JS. Peroxynitrite-induced cytotoxicity in PC12 cells: evidence for an apoptotic mechanism differentially modulated by neurotrophic factors. J Neurochem 1995; 65: 1543-1550.
116. Hunot S, Brugg B, Ricard D, et al. Nuclear translocation of NF-κB is increased in dopaminergic neurons of patients with Parkinson disease. Proc Natl Acad Sci USA 1997; 94: 7531-7536.
117. Patil N, Cox DR, Bhat D, Faham M, Myers RM, Peterson AS. A potassium channel mutation in weaver mice implicates membrane excitability in granule cell differentiation. Nature Genetics 1995; 11: 126-129.