Pathology, Aetiology and Pathogenesis

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Introduction

Despite intensive research activity over many years the cause of Parkinson's disease remains unknown. Major advances have been made, however, in our understanding of the mechanisms involved in neural degeneration and in neurotransmitter biology. The role of genetic factors has also been an area of considerable advance. It has been suggested that different presentation and progression of disease in young onset and elderly patients might indicate that these are different conditions. Apart from the possibility that genetic factors might be of more relevance in young onset patients, there is no evidence to suggest that the underlying pathology and pathophysiology differ with age of presentation. In this chapter I review the current state or our knowledge of the pathology, pathophysiology and aetiology of Parkinson's disease as it relates to our understanding of the clinical presentation and management of the condition.

Neurochemistry

Depletion of the neurotransmitter dopamine is the main neurochemical abnormality in Parkinson's disease. Other neurotransmitters affected include noradrenaline, serotonin and acetylcholine but the role of these substances is uncertain.

Almost 80% of brain dopamine is found in the striatonigral complex comprising the putamen, caudate and substantia nigra pars compacta (SNc). The SNc is the principal source of dopaminergic neurones. These project mainly to the putamen and caudate[1]. The extent of dopamine depletion in these structures correlates with neuronal loss in the SNc. In Parkinson's disease dopamine depletion is more evident in the putamen than caudate[2].

Dopamine is synthesised in the brain from the amino acid tyrosine. The first step is conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa), catalysed by the enzyme tyrosine hydroxylase. This is the rate-limiting step in dopamine production. Levels of tyrosine hydroxylase are low in Parkinson's disease explaining why tyrosine is ineffective as therapy. L-dopa is converted to dopamine by dopa decarboxylase (aromatic amine decarboxylase). Dopamine is stored in vesicles and released by a calcium dependent mechanism. After release, dopamine is removed from the synaptic cleft by an active reuptake mechanism, following which it is available again for vesicular storage. Dopamine is metabolised enzymatically by both intracellular monoamine oxidase (principally MAO-B) and extracellular catechol-o-methyl transferase (COMT)[3]. Homovanillic acid is the main metabolite.

Two families of dopamine receptor have been identified: the D1 family which includes D1 and D5 receptors and the D2 family which includes D2, D3 and D4 receptors. After binding to D1 type receptors dopamine acts via an increase in cyclic AMP whereas the effect on D2 type receptors is to decrease cyclic AMP[4]. D2 receptors are thought to be important mediators of the therapeutic effects of pharmacological agents, the role of D1 receptors being less clear.

Physiology of basal ganglia motor control

The defect in PD causes disruption to the dopaminergic nigrostriatal projections and interferes with function of the motor circuit of the basal ganglia. This circuit is involved in the control of both voluntary and involuntary movement. The function of the motor circuit is very complicated. Several subcircuits which interact in a complex manner, still poorly understood, are involved[5]. These include the interaction between the globus pallidus externa (GPe) and the subthalamic nucleus (STN) which is thought to be of particular importance. The following is a simplified version of current understanding of how motor activity is influenced by this circuit and how this function is altered in PD.

The key to understanding the function of the basal ganglia in controlling movement is the appreciation of the roles of the globus pallidus interna (GPi) and the venterolateral thalamus (VL). The VL has an excitatory output to the motor cortex and thus facilitates movement. The GPi, along with the substantia nigra pars reticulata (SNr), has an inhibitory output to VL and therefore acts as a 'brake'. This means that the effect of increased output from the GPi / SNr is to inhibit movement.

GPi receives input from the putamen via two pathways – direct and indirect. The direct pathway runs monosynaptically from putamen to GPi and its effects are inhibitory, i.e. to release the brake on movement exerted by GPi. The indirect pathway runs from the putamen via GPe and STN. STN has an excitatory effect on

GPi, mediated by glutamate[6]. The effect of the indirect pathway is therefore to increase the braking on movement from GPi.

Normal dopamine release from the substantia nigra pars compacta (SNc) acts on both these pathways, direct and indirect. By acting on dopamine D1 receptors, the direct pathway is stimulated to decrease the braking effect on movement from GPi. By acting on dopamine D2 receptors the indirect pathway is inhibited, decreasing the stimulus for GPi to exert a braking effect[7]. Normal SNc function therefore results in a low braking effect from GPi to VL and thus allows VL to facilitate movement via its excitatory effects on the motor cortex.

In PD decreased dopamine release from SNc disrupts this mechanism. Understimulation of the direct pathway and underinhibition of the indirect pathway result in an increased inhibitory ('braking') output from GPi to VL. Thus the excitatory effects of VL on the motor cortex are diminished and movement inhibited (*Diagram 1*).

Levodopa acts by increasing SNc dopamine output and therefore normalising basal ganglia function by stimulating both D1 and D2 receptors. Direct dopamine agonists vary in their differential effects on D1 and D2 receptors but all have significant D2 activity. The potential role of drugs active predominantly on the D1 receptors is still under investigation. The important consequences of abnormal increased activity of GPi and STN on motor function are the reason these areas are important target sites for neurosurgery whether by ablation or high frequency stimulation.

Pathology

The pathological changes of PD include cell loss in a specific distribution, the presence of Lewy bodies in surviving cells, and an undamaged striatum (comprising putamen and caudate).

Cell loss

The main area of cell loss is in the substantia nigra (SN). Cell loss also occurs outwith the SN in a widespread but specific pattern. Other areas affected include the noradrenergic locus coeruleus, the thalamus, the hypothalamus, the cholinergic nucleus basilis of Meynert, dopaminergic neurones of the ventral tegmentum, the serotonergic raphe nuclei, the limbic cortex and cerebral neocortex, and the autonomic nervous system (including sympathetic and parasympathetic ganglia and the myenteric plexus in the walls of the oesophagus and colon)[8; 9].

The SN is divided into the pars reticulata and the pars compacta (SNc). The SNc is predominantly affected. Cell loss in excess of 50% of normal levels is required for clinical symptoms to develop[10]. The SNc can be further subdivided into ventral and dorsal tiers. Cells in the dorsal tier contain more neuromelanin compared to the ventral tier. Neuromelanin is derived from the auto-oxidation of dopamine and accumulates throughout life. The increase in neuromelanin in the dorsal tier probably represents more active dopamine turnover compared to the paler ventral cells[11].

Cell loss in the SNc in PD preferentially affects the ventral tier[12]. By death about 23% of cells in the SNc remain compared to normal, but the surviving cells are mainly in the dorsal tier[9]. This is in contrast with normal ageing where the dorsal tier is mainly affected. In normal ageing the SNc shows an approximately 5% neuronal loss per decade after the age of 40. The loss is greater in the dorsal tier by a ratio of over 3:1[13]. This is evidence that PD is not simply an exaggeration of the ageing process.

The pathological changes seen in PD are more active that those seen in ageing. An increase is seen in neuronal fragmentation, extraneuronal melanin and gliosis over and above that seen age in age matched controls. Six times as many neurones are being actively phagocytosed compared with controls[14].

The functional consequence of nigral cell loss is the loss of normal nigrostriatal innervation and the clinical features of PD. As much as 80% of dopamine may be lost in the striatum by time clinical symptoms emerge. By death dopamine levels are reduced to 2% of normal in the putamen and 16% of normal in the caudate[9].

The consequences of cell death in other affected areas are less well understood. It is thought that damage in the cerebral cortex, nucleus basilis of Meynert, and the ventral tegmentum is associated with cognitive dysfunction. Although damage to the autonomic system can be linked to clinical features of autonomic failure, other factors including antiparkinsonian medication are relevant. The consequence of damage in other areas including the thalamus, locus coeruleus and raphe nuclei is unknown.

Despite the consequences of abnormal innervation from the SN, it is likely that no cell loss occurs in the striatum. This is in contrast to the marked striatal degeneration seen in other conditions such as multiple system atrophy (striatonigral degeneration), progressive supranuclear palsy, corticobasal degeneration and Huntingdon's disease[15].

Lewy bodies

Lewy bodies are neuronal inclusions always found in PD, occurring in all areas of neuronal degeneration. They consist of a central core which stains densely with haemotoxylin and eosin. This is surrounded by an area staining less densely and a peripheral halo staining lightly or not at all[8]. They contain neurofilament proteins along with several other proteins involved in proteolysis including ubiquitin and proteases[16]. Other constituents include tubulin and α-synuclein[17]. It has been suggested that the function of Lewy bodies is to eliminate damaged proteins from cells as part of a protective response to stress[18]. It is not known whether this is ever successful or whether the presence of a Lewy body indicates that the cell is doomed. Lewy bodies are not unique to PD. Other conditions in which Lewy bodies can be seen in degenerating neurones include corticobasal degeneration, progressive supranuclear palsy, motor neurone disease, ataxia telangectasia and Hallevorden-Spatz disease[19]. PD, however, is the only condition in which Lewy bodies are invariably found, occurring in only a proportion of these others. Cortical Lewy bodies are always found in PD.

Another cellular inclusion the 'pale body' is frequently but not always found in PD. They consist of round vacuolated granular areas within cell bodies[20]. They also stain positively for ubiquitin and neurofilament proteins and it has been suggested that they are a precursor to the Lewy body. Pale bodies have been described only in PD[15].

Isolated Lewy body disease

Lewy bodies and neuronal loss in the characteristic pattern of that found in PD can be demonstrated at post mortem in subjects with no clinical evidence of PD in life. This has been termed Incidental Lewy Body Disease (ILBD). The prevalence of ILBD is approximately 1% of the non-parkinsonian population dying in their fifth decade, rising to 10% of those dying in their eighth decade[19]. Cell loss is found in the ventral tier of the SNpc to a degree intermediate between that found in PD and in normal ageing. As well as typical pathology, ILBD shows low levels of reduced glutathione in the substantia nigra indicating oxidative stress (see below – pathophysiology). These findings are not a feature of normal ageing and ILBD is therefore regarded as preclinical PD.

Rate of progression of PD

There is no evidence to suggest that the rate of progression of PD is affected by age of onset[8]. The fact that 50 –60% of pigmented nigral cells are lost at the onset of symptoms yet this decreases to only 20% of normal after disease duration of 25 years suggests that cell loss is slow[13]. A 1% prevalence of ILBD at ages 50-59 corresponds to a 1% prevalence of PD at ages 80 –89. This suggests a 30 year delay before symptoms emerge[19]. Other evidence, however, from serial PET scans[21] and from post mortem cell counts in different disease durations have suggested a shorter preclinical phase of between 5–10 years[13].

Overlap pathologies

PD shares some clinical and pathological features with a number of other neurodegenerative conditions, in particular Dementia with Lewy Bodies (DLB) and Alzheimer's Disease (AD). The precise relationship between these conditions is unclear and remains controversial.

Dementia with Lewy bodies is the preferred term for a condition previously described as Lewy body dementia, the Lewy body variant of AD, senile dementia of Lewy body type or diffuse Lewy body disease[22]. The range of nomenclature reflects different interpretations depending on the range of clinical and pathological findings in cases studied. The condition is thought by some to be the second commonest cause of dementia after AD[16]. Pathologically DLB is characterised by the presence of cortical Lewy bodies. Cortical Lewy bodies differ from those seen in subcortical regions being less well defined[18]. Lewy bodies are also seen in subcortical structures including the substantia nigra. These subcortical changes are identical to those found in PD but can be mild. Most cases of DLB also have pathology which overlaps that of AD including plaques and neurofibrillary tangles[23]. There are however some differences in morphology and distribution of these features compared to AD. It is also the case that patients with PD who are cognitively intact invariably have a number of cortical Lewy bodies. These can be of a number and distribution which overlaps that seen in DLB[16].

There is also evidence of some overlap between PD and AD. Dementia is common in PD, occurring in a quarter to a third of patients[24; 25]. Likewise parkinsonism, principally bradykinesia and rigidity, is common in AD, again with rates of 33% and more being reported[26]. It is possible that these figures are an underestimate due to poor recognition of cognitive deficit by movement disorders specialists and of extrapyramidal features by psychiatrists. In both these conditions there is a progressive loss of specific neurones and the occurrence of intraneuronal inclusions. Plaques and neurofibrillary tangles are found in the brains of PD patients at postmortem up to six times more frequently than in controls, and in all cases of PD with severe dementia[27; 28]. Conversely, between 20% and 40% of AD patients show changes in the substantia nigra at postmortem consistent with a diagnosis of PD[29; 30]. Cases of clinically typical, levodopa-responsive PD have been described showing neurofibrillary tangles in the substantia nigra but no Lewy bodies[31].

There is therefore considerable pathological overlap between these conditions including the presence of Lewy bodies, plaques and neurofibrillary tangles. It has been suggested that there is a spectrum of Lewy body disorders with PD at one extreme and DLB at the other. Some have gone further suggesting a broader spectrum of disease ranging from PD to AD with DLB as an intermediate condition[23]. Other possible interpretations are that these are distinct conditions sharing final common pathological pathways or that the coincidence of pathology represents common genetic risk factors for these conditions.

Aetiology

A major problem in defining aetiological factors in PD is the presence of a large amount of preclinical disease. The overall age-adjusted prevalence of ILBD is 5.6% compared to a typical prevalence of clinical PD of 0.2%[32]. ILBD is therefore many times more common than clinical disease. Most individuals with ILBD will never go on to manifest clinical PD perhaps because they do not live long enough for this to become apparent[19]. It has been postulated that clinical PD represents the youngest 5-10% of a virtual bell-shaped distribution curve of incidence that has a maximum at an age approaching 175 years[32]. Thus only a small percentage of individuals with PD pathology are open to study in a search for aetiological factors. Furthermore there might be important differences in these subjects with respect to risk factors compared to the majority with ILBD.

Bearing this caveat in mind there follows a discussion of the roles of ageing, genetic influence and environmental risk factors in the development of PD and the pathophysiological processes involved.

Ageing

Advancing age is the single most important risk factor for developing PD. Prevalence rates for PD in epidemiological studies typically show an exponential increase from 1 per thousand in the general population to as much as 2% in those over 80 years of age[33]. It is difficult to define what 'normal' ageing of the CNS is. It is unclear to what extent the changes seen are physiological or represent the accumulated effects of pathological processes. Nonetheless a number of changes are recognised to occur with ageing. These include a decrease in the numbers of pigmented neurones in the substantia nigra[34], a decrease in striatal dopamine[35], a decrease in striatal tyrosine hydroxylase (involved in dopamine synthesis)[36], and a decrease in dopamine receptors[37]. As previously discussed, however, the distribution of neuronal loss within the substantia nigra is different from that seen in PD. The exact role of ageing in pathogenesis is not clear but it does not appear that PD is caused by an exaggeration of normal ageing processes. It is likely that age-related changes combine with other pathological mechanisms to produce the clinical syndrome[38].

Genetics

Genetic studies are difficult t carry out in PD. Evidence for a genetic role in the aetiology of PD has come from a number of sources including twin, family, case control and epidemiological studies. Each has its problems mainly due to the absence of a good marker for the condition and the subsequent difficulty in case ascertainment.

Genetic disease can be either single gene and Mendelian or due a complex interaction of multiple genes in a non-Mendelian manner. The latter almost certainly accounts for the majority of genetic influence on PD but a number of major advances have taken place in recent years in describing the role of single gene defects in causing familial PD.

Family studies

Most patients with PD do not have a family history and it is highly likely that non-genetic factors are important in aetiology. Nonetheless, family history is the next most important risk factor for PD after age. A positive family history can be obtained in 20 – 30% of patients with PD[39]. This rises to a positive history in 43% versus 9% of controls if a history of tremor alone is included[40]. In case control studies the relative risk for a first-degree relative of developing PD is around 3.5[41]. This evidence provides strong support for the role of genetic factors on the development of PD.

Twin studies

Early twin studies in PD failed to demonstrate a significantly increased concordance rate in monozygotic compared to dizygotic twins and were interpreted as evidence that genetic factors were not relevant. The studies were potentially flawed due to the fact that PD could only be identified if it had become clinically apparent in a sibling and a failure to recognise atypical presentation e.g. isolated tremor as a *forme fruste* of PD. Later, however, using PET scans have demonstrated abnormal flurodopa uptake in apparently unaffected siblings. Concordance rates of 45% in monozygotic versus 29% in dizygotic twins have been reported[42] but the results were inconclusive. A recent study, again using flurodopa PET scanning to identify asymptomatic disease has shown concordance rates rising to 75% in monozygotic twins compared to 22% in dizygotic twins over 7 years follow up. This suggests a significant role for inheritance in the development of sporadic PD[43].

Epidemiology

Epidemiological studies have shown that the prevalence of PD is not uniform throughout the world. A number of studies have shown that populations moving from a low prevalence area to one in which there is a higher prevalence of PD, gradually acquire the same prevalence as the 'host' country. For example the prevalence of PD in Nigeria is much lower than amongst African-Americans in the U.S.A. despite a fairly homogenous genotype[44]. This is good evidence for an environmental factor in the causation of PD but such studies must be interpreted with some caution. There are a number of possible sources of error. It is difficult to be sure of the true prevalence in the country of origin particularly if this is a Third World country with poorly developed medical services. Cases of PD may not be reliably diagnosed giving a falsely low prevalence. It is also possible that, in some cases, migrants are not typical of the population at large and do not share the same predilection to develop PD.

Single gene defects as a cause of PD

Golbe et al in 1990 described a pedigree of an Italian American family in which parkinsonism was inherited as an autosomal dominant condition[45]. Clinically the disease was consistent with idiopathic PD but there was a tendency for an earlier age of onset and a rather more aggressive course than usual. Post mortem studies have confirmed the presence of typical pathology with Lewy bodies. Subsequent investigation by Polymeropoulos et al showed that the problem lay in a mutation of the α-synuclein gene on chromosome 4[46]. The same mutation has subsequently been identified in 3 Greek families and a second mutation in a German family[46; 47]. The biological role of α-synuclein is unknown. It is a protein found in cell nuclei and in nerve terminals (thus the name). It is found in a wide range of species. It is known to be expressed in young birds whilst learning to sing and it has therefore been suggested that it has role in neuronal plasticity[48]. It is interesting to note that Lewy bodies in idiopathic PD (without the α-synuclein mutation) stain more positively for α-synuclein than for ubiquitin[17]. α-synuclein is now the best marker for Lewy bodies but the role of the protein in pathogenesis is unknown. It has been postulated that the mutation results in an abnormality in folding of the protein causing it to accumulate[49].

Since the report of this gene mutation a number of groups have searched for its occurrence in kindred with familial PD. No other cases have been found in a large European study of familial PD[50] or in a British study of sporadic PD[51] and it is clear that the α-synuclein mutation is a very rare cause of PD.

More recently another single gene defect causing parkinsonism has been described – the 'parkin' gene. Although clinically similar to idiopathic PD, Lewy bodies are not seen at post mortem. This was first described by a group of Japanese workers as a cause of juvenile parkinsonism inherited as an autosomal recessive[52]. This gene has been found in a number of other kindred in Europe and elsewhere and may be a relatively common cause of familial parkinsonism presenting before the age of 58[53]. The protein product of the parkin gene has a homology to ubiquitin and is present in brain and substantia nigra. The mechanism whereby loss of this protein relates to neurodegeneration is unknown.

Other single gene defects are under investigation but an emerging pattern is of younger onset disease that in sporadic PD.

'Candidate' studies

The developments in describing single defects as a cause of PD have been exciting and valuable in providing clues to pathophysiology. These single gene defects, however, do not account for the majority of sporadic cases of PD. Here inheritance is likely to be determined by a number of genes interacting in a complex manner. Rather than search randomly in the genome for genes associated with PD a more rapid approach may be to use our knowledge of the pathology and factors involved in pathophysiology of PD and to target genes known to have a biological action which might be relevant – so-called 'candidate' studies. Genes involved in dopamine metabolism, oxidation reactions and in detoxification have been examined. Results have been inconsistent and no clear linkage with PD has been demonstrated. Recently, it has been found that the slow acetylator genotype for N-acetyltransferase-2 is more common in familial PD[54]. This has prompted the hypothesis that there may be an impaired ability to handle neurotoxic substances.

Environmental factors

A great deal of effort has been made to find environmental factors causing PD. Toxic substances have been found which cause a parkinsonian syndrome similar but not identical to idiopathic PD. A number of factors that appear to be associated with an increased risk of PD have also been described. Evidence for the important role of environmental factors comes for the USA Veteran Twin Study[55]l. In this study the ratio of PD in monozygotic twins was 1.06 in those with disease onset greater that 50 years of age. This implies that genetic factors are unlikely to be important. In contrast, for those with disease onset under 50 years the ratio was 6.00 in favour of the monozygotic twin implying a more important role for genetic factors. It is therefore likely that environmental factors are important in the development of PD and become more important with increasing age of disease onset. It is likely that the less 'genetic' the susceptibility to PD, the more age and environment need to contribute to the development of disease[56].

Specific toxins

Manganese causes an akinetic rigid syndrome in man, predominantly due to a toxic effect on the globus pallidum and striatum. The mechanism of this toxic action is unknown. There are clear clinical differences, however, from PD[57]. Other metals including copper and iron have been shown to be increased in the substantia nigra but this is probable a secondary phenomenon and not the primary cause of PD[39]. In survivors of poisoning, carbon monoxide causes parkinsonism developing after a few days or weeks by necrosis of the globus pallidum[58].

The discovery that the 'designer drug' contaminant 1-methyl-4phenyl 1,2,3,6 tetrahydropyridine (MPTP) could cause an acute parkinsonian syndrome has proven to be an extremely valuable clue as to the pathogenesis of PD[59]. A number of individuals who repeatedly injected themselves with this agent developed an akinetic rigid syndrome within days. The clinical syndrome is very similar to idiopathic PD.

There is a response to levodopa and typical complications of therapy including fluctuations and dyskinesias develop[60]. Lewy body pathology is not seen, however, and the syndrome cannot therefore be regarded as identical to idiopathic disease. MPTP is a protoxin. It is converted to the toxic metabolite 1-methyl-4-phenylpyridium ion (MPP^+). This compound is actively taken up by the dopamine reuptake system and concentrated in dopaminergic neurones. Once in the cells MPP^+ inhibits interferes with mitochondrial function and thus cellular energy production. The specific site of action is Complex 1 of the mitochondrial respiratory chain. MPP^+ is also a generator of free radicals – another mechanism for neurotoxicity[58]. MPTP causes acute parkinsonism in other primates and has allowed the development of animal models for PD.

The discovery of MPTP stimulated a search for other toxic substances in the environment as possible causes of idiopathic disease. High on the list of suspects have been pesticides and herbicides particularly as MPP^+ is chemically related to paraquat, a previously commonly used herbicide. This hypothesis would fit well with a reported increase in PD in association with rural living (see below). Against this, however, is the fact that the prevalence of PD has not risen since the widespread introduction of these chemicals[39].

Other risk factors

A number of studies have described a relationship between rural living and the development of PD[61; 62]. The evidence, however, is contradictory with other studies showing no such association[63]. A number of factors have been suggested to explain this. These include well-water drinking and exposure to agricultural chemicals.

The possibility that oxidative mechanisms are relevant for the pathogenesis of PD (see below) has prompted examination of dietary factors. An association with increased animal fat consumption has been described[64]. The role of antioxidants including vitamins E and C have been examined for a potentially protective role but no clear consensus has emerged. Vitamin E supplementation is likely to have no effect on the progression of PD[65].

Cigarette smoking has been repeatedly shown to be associated with a decreased risk of developing PD[39]. Non-smokers have generally been shown to be twice as likely to develop the disease. This effect remains even after allowing for differences in mortality. Suggested mechanisms include the fact that nicotine causes dopamine release and up regulation of dopamine receptors, potentially masking signs of early or mild disease. Cigarette smoke contains a monoamine oxidase B (MAO-B) inhibitor[66]. This might be relevant for a number of reasons. MAO-B inhibition decreases dopamine break down and could therefore boost levels in the brain. Reduced free radical production associated with this action on dopamine could also protect against oxidative stress. Finally MPTP induced parkinsonism can be ameliorated by MAO-B inhibition by preventing conversion to MPP^{+}[67].

Not all studies have confirmed the protective effect of smoking. It has been pointed out that no dose-response relationship, which would be expected, has been shown.

Others have suggested that the relationship might be the other way round i.e. PD might reduce smoking perhaps due to psychological factors[39].

Pathogenesis

Apoptosis

Whatever the cause or causes of PD, it is likely that nigral cell loss occurs via a common pathophysiological pathway leading to apoptosis (programmed cell death). Cells can die either by necrosis or apoptosis. In necrosis an external insult is responsible for death whereas in apoptosis cell death occurs as a result of an intracellular process regulated by genes. Apoptosis is well documented as a normal physiological process in the development of the nervous system[68]. More recently its pathological role in neurodegenerative disorders has been recognised. The identification of apoptosis depends on finding specific morphological changes including chromatin clumping[69]. This is technically difficult and there is still some controversy over whether apoptosis is important in PD[70; 71]. There is, however, a developing consensus that changes of apoptosis can be identified in the substantia nigra pars compacta (SNc) at post mortem. The number of apoptotic nuclei in the SNc in PD at 2% is approximately ten times that seen in normal ageing[56].

A number of processes interact to cause apoptotic cell death in the SNc in PD. These include oxidative stress, mitochondrial dysfunction and excitotoxicity.

Oxidative stress

The cells of the SNc are particularly vulnerable to oxidative damage due to the presence of dopamine, neuromelanin, and high levels of iron[56; 58]. Dopamine undergoes oxidative deamination (mediated by monoamine oxidase). In addition dopamine is prone to auto-oxidation. These processes yield metabolites including hydrogen peroxide. Neuromelanin binds ferric iron and can reduce it to its reactive ferrous form. This process facilitates the conversion of hydrogen peroxide to oxyradicals including the highly toxic hydroxyl radical. Other products of dopamine auto-oxidation include the superoxide radical (***Diagram 2***).

Increased iron levels also occur in other neurodegenerative diseases including Multiple System Atrophy and Progressive Supranuclear Palsy. These conditions are also associated with cell loss in the basal ganglia. Increased iron is not seen in ILBD. It is probable therefore that this is a secondary and late phenomenon. Nonetheless it is might still be of importance as it could form part of the cascade leading to cell death[58; 72].

In normal cells there are a number of defence mechanisms to protect against oxidative damage. These include scavenger enzymes including catalase and peroxidase. Important in this process is the presence of reduced glutathione (GSH). Levels of GSH have been shown to be low in the substantia nigra in PD suggesting increased free radical generation. Low nigral glutathione levels are also found in ILBD but not in other parts of the brain or in other neurodegenerative diseases. It is possible therefore that this is an important mechanism early in the development of PD[58; 72].

There is direct evidence for oxidative damage in the substantia nigra in PD. Levels of malondialdehyde are increased indicating increased lipid peroxidation[73]. In addition 8-hydroxy-2-deoxyguanosine is increased indicating oxidative damage to DNA[74].

Mitochondrial dysfunction

Mitochondria play a crucial role in cellular energy production. They produce ATP by oxidative phosphorylation. The mitochondrial respiratory chain consists of five protein complexes. Complex 1 is deficient in the substantia nigra in PD by approximately 35%[58]. The deficiency is specific to complex 1 with other proteins in the respiratory chain unaffected. It is also site and disease specific. Other parts of the brain appear to be unaffected and the deficiency is not found in other degenerative diseases such as multiple system atrophy which show a similar nigral cell loss[56]. A modest (20 – 25%) reduction in Complex 1 activity can also be demonstrated in the platelets of patients with PD[75]. This, however, is not sufficiently sensitive to be used as a biological marker for the condition.

It has recently been suggested that mitochondria might have a critical role in the sequence of events leading to apoptosis. A decrease in mitochondrial membrane potential and increased intramitochondrial calcium appear to be important early events in this process. These lead to the opening of a mitochondrial pore and the release of apoptosis initiating factors[56].

Mitochondrial dysfunction and oxidative stress may interact to reinforce the toxic effects of each. It is postulated that complex 1 deficiency is associated with superoxide ion generation, increasing oxidative stress. This in turn might worsen the complex 1 defect in a vicious spiral of toxicity[58].

Excitotoxicity

The striatum contains widespread glutaminergic projections acting on NMDA receptors. Glutamate is an excitatory neurotransmitter with the potential to cause cell damage via excitotoxicity. Glutaminergic stimulation is mediated by an influx of calcium ions into the cell. If excessive this can cause toxicity via activation of a variety of enzyme systems[76]. Normally excessive calcium influx is blocked by magnesium ions within the receptor ion channel. This ion channel blockade is dependent on the ability of the cell to maintain a normal membrane electrical potential. This in turn is dependent on mitochondrial ATP production. Mitochondrial dysfunction in PD may thus cause decreased magnesium blockade and expose the nigral cells to excitotoxicity from excessive calcium influx[77]. In PD physiological levels of glutamate stimulating the NMDA receptors could be toxic further increasing mitochondrial damage and oxidative stress[56] (*Diagram 3*).

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Diagram 1a: The normal motor circuit.

Diagram 1b: The motor circuit in Parkinson's disease.

White arrows represent excitation and black arrows inhibition

Key: SNc = substantia nigra pars compacta, GPe = globus pallidus externa, STN = subthalamic nucleus, GPi = globus pallidus interna, SNr = substantia nigra pars reticulata, VL = venterolateral thalamus

Diagram 2: Oxidative mechanisms and free radical production.

Diagram 3: Pathogenesis of Parkinson’s disease