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Etiology of Parkinson's Disease: A Research Strategy

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SUMMARY: In this essay I present a new "global approach hypothesis" to explain the pathophysiology of Parkinson's disease: "Susceptibility to Parkinsonism is genetically determined and is reflected in all cells. I propose that idiopathic Parkinson's disease is the combined result of a generalized cell aging process accelerated, in susceptible individuals, by a variety of often repetitive trigger factors. These factors have in common the fact that they cause a transient increase in turnover within catecholamine producing neurons, centrally as well as peripherally. This results in accumulation within these neurons of free radicals. When the level of the toxic substances, in quantity or in time of exposure, exceeds the scavenging capacity of the cell, damage to organelles and to membranes results, leading to the formation of Lewy bodies through an autoimmune reaction to damaged filaments and to cell death, particularly in the pigmented neurons of the brainstem. The progressive cell depletion leads to a compensatory increase in catecholamine turnover in the remaining pigmented cells, and an ever-accelerating degenerative process. The resulting neurotransmitter imbalance in the basal ganglia explains the symptoms of Parkinson's disease". In the light of this hypothesis, our research objectives should be (1) to delineate the limits of true Parkinson's disease from all phenocopies; (2) to identify individuals susceptible to parkinsonism and the most common trigger factors; (3) to reduce the metabolic effects of unavoidable trigger factors and (4) to protect susceptible individuals by increasing the functional availability of free radical trapping agents.

RÉSUMÉ: Dans cette communication je présente une nouvelle hypothèse globale sur la physiopathologie de la maladie de Parkinson: "La susceptibilité au Parkinsonisme est déterminée génétiquement et se reflète dans toutes le cellules. Je propose le concept que la maladie de Parkinson idiopathique est en fait le résultat combiné d'un processus de vieillissement généralisé mais accéléré, chez des individus susceptibles, par une variété de facteurs déclanchants souvent répétitifs. Ces factuers ont en commun le fait qu'il causent une augmentation transitoire du turnover dans les neurones catécholaminergiques, centralement et en périphérie. Ceci produit, à l'intérieur de ces neurones, une accumulation de radicaux libres. Quand le niveau de ces substances toxiques, tant en quantité qu'en durée d'exposition, dépasse la capacité vidangeuse de la cellule, il en résulte des lésions aux organelles et aux membranes, lésions allant jusqu'à la mort cellulaire, surtout dans les noyaux pigmentés du tronc cérébral. La perte céllulaire progressive conduit à une augmentation compensatrice du turnover des catécholamines dans les neurones pigmentés survivants et donc à neurotransmetteurs dans les noyaux gris centraux, qui lui est secondaire, explique les symptômes de la maladie de Parkinson". A la lumière de cette hypothèse, nos objectifs de recherche sont (1) de délimiter le cadre nosologique précis de la véritable maladie de Parkinson et de la séparer des nombreuses phénocopies. (2) d'identifier les individus susceptibles au Parkinsonisme et les facteurs déclanchants les plus usuels (3) de réduire les effets métaboliques des facteurs déclanchants inévitables et (4) de protéger les individus susceptibles en augmentant la disponibilité fonctionnelle d'agents vidangeurs des radicaux libres.

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Despite great strides in the last 20 years which have considerably improved the functional outlook of patients suffering from Parkinson's disease, and despite the demonstration of a crucial dopamine deficiency, we are not much closer to understanding the cause of this disorder. A number of hypotheses have been proposed, exploring the avenues of virology, immunology, toxicology and genetics, but none of the above concepts has proven completely satisfactory when taken alone, possibly because *all* of these factors have *some* role to play, but without exclusivity, in the etiology of Parkinson's disease. The incidence of the disease is known to increase considerably with age, thus it is not surprising that the more lasting hypothesis has been the proposal for some form of "accelerated aging phenomenon" (Barbeau, 1973), a concept supported by the most elegant studies of Mann and Yates (1974 a, b; 1977, 1978, 1979, 1982, 1983). However even this proposal cannot, by itself, explain why Parkinson's disease will affect only 1 in 40 persons over the age of 65.

In evaluating etiologic hypotheses in Parkinson's disease, one must first get rid of a common misconception. It is usually stated that the pathology of this disease is characterized by "depigmentation of the substantia nigra", and this is often understood to mean that there is a defect in the process of melanization. Because tyrosine hydroxylase is the rate-limiting step in the synthesis of catecholamines, this enzyme was implicated by many authors, particularly after the demonstration of low CSF levels of its co-factor tetrahydrobiopterin. However there is evidence that the decrease in tyrosine hydroxylase activity is directly proportional to dopamine cell loss in the substantia nigra and is thus a secondary phenomenon. The

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correct pathological statement should be that the substantia nigra of parkinsonian patients contains fewer live pigmented cells, thus giving a paler overall gross appearance to the nuclei. Indeed, once the cells die, the melanin pigments are phagocytosed and carried away to nearby vessels. The basic defect in Parkinson's disease therefore resides in the mechanism of preferential cell death in the nuclei of the brain stem, not in the process of melanization. A valid hypothesis for the etiology of Parkinson's disease must explain this preferential cell death as well as the almost universal appearance of Lewy bodies in the pigmented cells.

To the difficulties in determining the etiology, should be added the problems concerning the delineation of the so-called "Idiopathic Parkinson's disease" from a host of entities imitating one or more of the cardinal symptoms (tremor, rigidity or akinesia). These entities are progressive supranuclear palsy, normotensive hydrocephalus, Shy-Drager syndrome, olivopontocerebellar degeneration and the Parkinson-dementia syndrome. Many of these disorders could be grouped under the very useful term "Parkinsonian Multiple System Atrophies" proposed by Ropper (1983), and their etiology should not be confused with that of true Parkinson's disease.

Moreover, it should be remembered that it is very unlikely that all cases with such diverging symptomatology as (1), a rapidly progressive bilateral akinesia in a relatively young person or (2), a slowly progressive unilateral tremor with little rigidity and no akinesia in a 65 year old victim, are the very same disease. Much more work remains to be done on the clinical delineation of the various syndromes because proper investigations will only be feasible on completely homogeneous entities. Even after such clarification, what is needed above all, is a *comprehensive research strategy*. On the occasion of this important Canadian Congress of Neurological Sciences, I would like to present my own working hypothesis to the scrutiny of my colleagues.

The Hypothesis

"Susceptibility to Parkinsonism is genetically determined and is reflected in all cells. I propose that 'Idiopatic Parkinson's disease' is the combined results of a generalized cell aging process, accelerated in susceptible individuals by a variety of often repetitive trigger factors. The factors have in common the fact that they cause a transient increase in turnover within catecholamine producing neurons, centrally as well as peripherally. This results in the accumulation within these neurons of large quantities of free radicals or semiquinones. When the level of the toxic substances in quantity or in time of exposure, exceeds the scavenging capacity of the cell, damage to organelles and to membranes results. This leads to formation of Lewy bodies through an autoimmune reaction to damaged filaments and to cell death, particularly in the pigmented neurons of the brain stem. This progressive cell depletion is accompanied by a compensatory increase in catecholamine turnover in the remaining pigmented cells, and an ever-accelerating degenerative process". An outline of this hypothesis is illustrated in fig. 1.

The Evidence

In this short review I will list only the principal arguments in favour of my hypothesis. Details of the experiments will be reported elsewhere:

a) Genetic susceptibility

The two best published epidemiological studies on Parkinson's disease (Martin et al., 1973; Kondo et al., 1973) took into

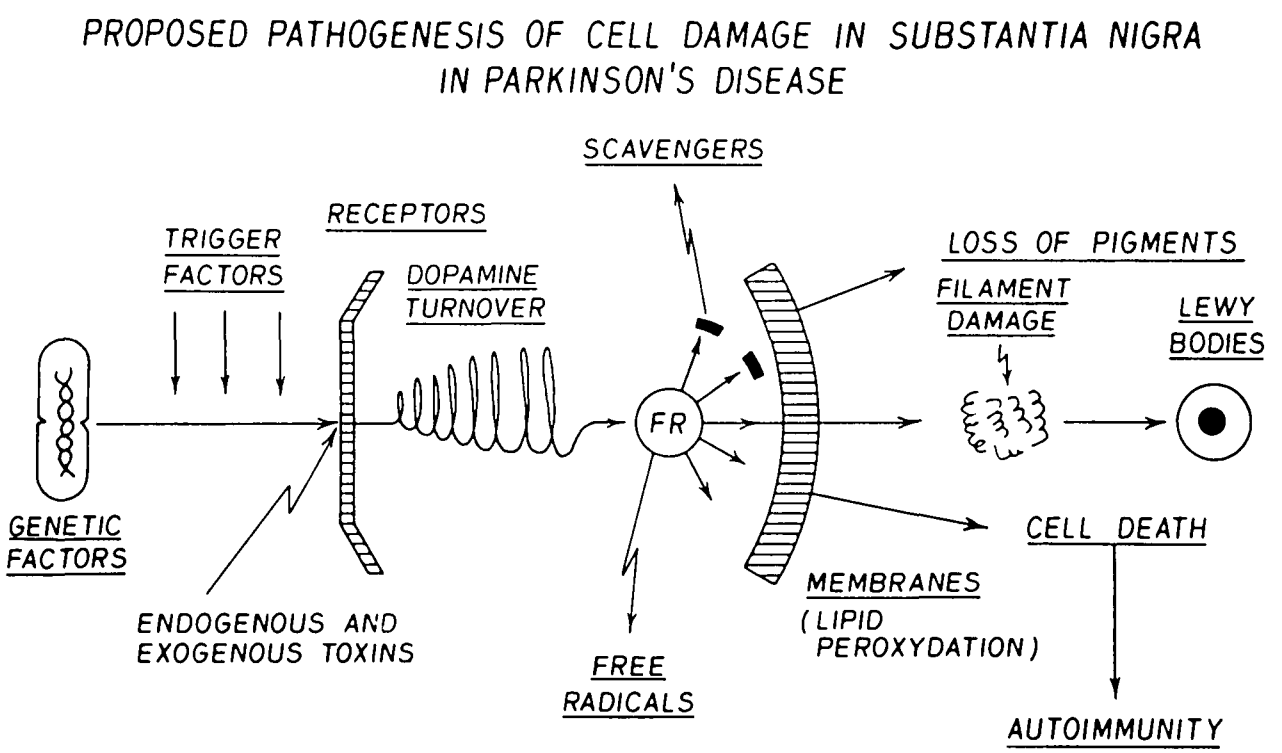

Figure 1 — Outline of our "global hypothesis" on the pathophysiology of Parkinson's disease. Details in text.

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account the difficulties of diagnosis and the need to examine all secondary cases in a finite population. Both these investigations similarly concluded that idiopathic Parkinson's disease is not a clear-cut genetic entity but that genetic factors play a role in its development. The proposed genetic model that best fits the available data is that of "multifactorial inheritance". This model generally implies the presence of one or more inherited "susceptibility factors" as a background to the effect of environmental agents. Another possible explanation for the observed 10-15% of "familial" cases, and one that we have explored (Barbeau and Pourcher, 1982; Roy et al., 1983; Barbeau and Roy, 1984), is that this agglomeration reflects the presence of one or more familial sub-sets of patients within the general group. In this way we have described an "essential-tremor related parkinsonism" and an autosomal recessive "familial akineto-rigid syndrome".

In the non-familial forms of Parkinson's disease, the role of "susceptibility factors" cannot be denied. Many years ago the incidence of basal ganglia disorders was found to be higher in relatives of patients developing extrapyramidal side-effects after neuroleptic treatment. A similar observation was made amongst relatives of miners exposed to manganese dust intoxication. If this "susceptibility" hypothesis is true, common disease-causing factors should eventually be uncovered. A number of authors have explored this possibility through the study of HLA polymorphisms, and their conclusions have been generally negative, no HLA sub-type being associated with Parkinson's disease in a constant manner. In a recent study, however, Barbeau and Roy (1984) uncovered another possible mechanism of susceptibility: the absence or decrease in the incidence of common HLA haplotypes normally thought to confer a biological advantage. Thus our parkinsonian patients had a much decreased incidence of the haplotypes A_1B_8 and/or A_2B_5. One still does not know how the decrease in these "protective factors" leads to specific enzymatic or membrane defects that predispose the cell to early death when it is exposed to toxic factors.

b) Trigger events

If Parkinson's disease is viewed as the result of environmental factors acting upon a genetically susceptible background, it is important to study such "trigger factors". The immediate problem is the multiplicity of such factors that are soon uncovered: trauma, toxins, and a variety of "*stress situations*" (defined as a period exceeding 2 weeks of emotional, situational, physical or economic upheaval), ranging in severity from a severe reducing diet to death of a close relative, loss of a job, marital difficulties, surgery (particularly to the gall bladder). More than one factor occurred in many subjects. 98% of our patients (and only 68% of our control subjects) had one or more episodes of identifiable disturbance to their health or well being within the 3 year period preceeding the onset of their illness. Interestingly, and possibly relating to an immunological anomaly, 40% of our patients recorded the occurrence of a "bursitis" to the shoulder or elbow in the three years preceeding onset. Only 8% of our age-matched controls were similarly affected.

However, the so-called "trigger factors" that can be readily identified in parkinsonian patients are mostly the same events which mark the unfolding of all of our lives. Although we cannot comment on their intensity, it is apparent that these events are only slightly more frequent in the patients. It is thus necessary, as seen above, to postulate an increased vulnerability of the target tissue to a common mechanism of action.

What is this common mechanism of action in all "stressful" situations? I postulate that it is an *increase in the turnover of catecholamines*, dopamine as well as noradrenalin, in central and peripheral nervous tissues. The evidence for this mechanism is well known and need not be reviewed here.

Suffice it to say that in Parkinson's disease this increased turnover is translated into a relatively higher level of basal ganglia HVA than dopamine at autopsy, and in the increased formation of urinary radioactive HVA after the intravenous injection of radioactive dopamine (Hornykiewicz, 1973; Barbeau and Trombitas, 1967).

Increased turnover of catecholamines, which can result from interference with pre-and post-synaptic receptors, or from modifications in the feedback control of various enzymes in the synthetic and metabolic pathways, is always accompanied with the production of large amounts of *free radicals* (Dormandy, 1983). Thus I propose that the common mechanism of all identified "trigger events" is the production of an increased turnover of catecholamines, with the result that cells implicated in the production of these amines are inundated by free radicals, at least transiently. This mechanism is operative in normal subjects as well as parkinsonians, thus it is necessary to postulate some special *vulnerability*, or fragility, of the substantia nigra and locus ceruleus cells in the diseased patients. This could be due to damage to the cell membranes, to normal aging or to a decrease in the normal protective mechanism of "scavenger enzymes" or antioxidants. There is some evidence for all of these possibilities.

c) Vulnerability

The involutional phenomenon of aging is best defined as a state of decreased viability and increased vulnerability. One of the main generalizations that can be made is that the process of aging is manifested on the histological level by atrophy of cells, particularly of nerve cells, with a compensatory increase in connective tissues, and often the deposition of pigments. Both lipofuscin and neuromelanin are known to increase with age, while in the aging brain, iron, copper, and calcium accumulate in excess. The increase in tissue iron can reach 200% in some species, mostly in glial cells. The iron accumulation and cytosiderosis reported in parkinsonian brains is an argument for some form of accelerated aging process (Barbeau, 1969).

Although parkinsonian patients may have evidence of generalized accelerated aging, it it most likely that this mechanism is operative at selective or preferred sites: the substantia nigra, the locus ceruleus, the dorsal nucleus of the vagus, the inferior olive and dentate nucleus and the basal nucleus of Reichert. It is of interest that many of these areas are pigmented and are involved in the synthesis and regulation of specific monoaminergic fiber systems. Thus accelerated aging is the background upon which play the environmental and endogenous trigger factors.

d) Environmental Trigger Factors

In addition to the common and relatively unspecific "stress" factors, with their translation into increased turnover of catecholamines, which we have surveyed to now and which could be called "endogenous" in their mechanism, it is possible to produce lasting and damaging increases in free radicals from purely "exogenous" causes. Two examples come to mind:

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manganese (Mn) intoxication and intoxication with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).

We have reviewed in detail elsewhere the mechanism of action of manganese in producing Parkinson's disease (Barbeau, 1983), and have shown that free radical production is again the main mechanism of damage, for manganese, as it is for 6-hydroxydopamine and for dopamine itself. In this we confirm the important studies of Graham et al. (1978, 1979) and of Donaldson et al. (1982).

MPTP, a by-product in the production of a number of meperidine derivatives, including some with hallucinogenic properties, has received considerable attention since the report of Langston et al. (1983) which recalled the previous paper of Davis and collaborators (1979). In both these papers a chronic, irreversible, parkinsonism was produced in human subjects receiving the compound by injection. A further patient has now been found (Langston and Ballard, 1983) who was a chemist only handling the drug. The same compound has now been used to produce a primate model of parkinsonism (Burns et al., 1983). The lesion is selective for the substantia nigra and it appears that the compound first enters the neuron at the synapse and then moves to the cell body where it causes damage by production of free radicals. It is not yet known whether MPTP itself, or one of its metabolites is the culprit. The formation of the oxide from the double-bond is the probable site of action.

Although it is unlikely, except in drug addicts and chemists directly exposed, that Parkinson's disease is generally caused by MPTP, it is certainly possible that many people are constantly being exposed to products with a similar conformation found frequently in nature or industrially. Many herbicides, for example, including the well known Paraquat, may have a similar chemical mode of action. In susceptible individuals such products could initiate the process of cell degeneration found in Parkinson's disease. This field deserves thorough scrutiny.

e) Scavenger Enzymes

Free radicals will damage a cell if their concentration at any given moment exceeds the scavenging capacity of the protective mechanisms of the cells. Enzymes such as catalase, glutathione peroxidase and superoxide dismutase are very active in this regard, particularly in dopamine neurons. It is thus of interest that their concentration has been found decreased in the substantia nigra of parkinsonian patients, first by Ambani et al. (1975) and more recently by Perry et al. (1982). However, it is still unknown if this defect is primary (genetic) or secondary to inhibition by toxic factors. Preliminary studies in my laboratory (Poirier and Barbeau, 1983 unpublished) indicate that these enzymes are all in normal concentration in peripheral tissues of parkinsonians, thus probably eliminating a genetic defect.

f) Cell damage

Free radicals, and toxins indirectly acting through this mechanism, will damage cells and cell organelles in many ways. Free radicals will attack cell membranes through peroxidation of poly-unsaturated fatty acids such as linoleic acid. They will also tend to polymerize filaments and other proteins which then may be released and recognized as non-self, thus generating autoimmune mechanisms, and possibly the formation of Lewy bodies. These inclusions have indeed recently been found to contain neurofilament antigens (Goldman et al., 1983). The final mechanism of interference is through modification of the cell methylating system which transforms phosphoethanolamine to phosphatidyl choline and therefore insures the normal fluidity of the membrane. Each and everyone of these mechanisms could be operative in the face of increased catecholamine turnover and free radical production. Moreover these processes could contribute to the normal accumulation of neuromelanin in the substantia nigra cells. Any modification in the cytotoxic effects of this melanin accumulation would result in cell loss, preferentially of the already highly pigmented cells (Mann and Yates, 1982).

g) End result

Cell loss in the substantia nigra and locus ceruleus is soon translated into a specific loss of the catecholamines produced in the needed areas such as the striatum. The dopamine deficit, in particular, is then directly responsible for some of the symptoms of Parkinson's disease, particularly akinesia (Hornykiewicz, 1973). Because of this deficit, at the level of the effector cells, feedback mechanisms will soon enter into action and command a further increase in dopamine synthesis, and turnover, leading to further free radical production and cell damage. Thus it can be seen that the process soon becomes *self sustaining*, and a degenerative and progressive disease of the catecholaminergic systems is born!

Conclusion

I have presented a hypothesis on the mechanism of cell damage to the substantia nigra in Parkinson's disease. Although I have presented preliminary evidence for each of the steps proposed, it is evident that the scheme is much more a blueprint for a "strategy for research into the cause of Parkinson's disease" and that the hypothesis must now be tested step by step before it can be accepted. However, if the concept proves acceptable, some immediately obvious therapeutic and preventive approaches to the disease are delineated. Our research strategy should be: (1) to delineate the limits of true Parkinson's disease from all phenocopies; (2) to identify individuals susceptible to parkinsonism and the most common trigger factors; (3) to reduce the metabolic effect of unavoidable trigger factors and (4) to protect susceptible individuals by increasing the functional availability of free radical trapping agents.

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