### Biochemistry of Parkinson's Disease with Special Reference to the Dopaminergic Systems

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### **Abstract**

The cardinal neurochemical abnormality in Parkinson's disease is the decreased dopamine content in the striatum, resulting from the loss of dopaminergic neurons in the mesencephalon. Precise analysis of the dopaminergic neurons in the midbrain demonstrates, however, that this cell loss is not uniform. Some dopaminergic cell groups are more vulnerable than others. The degree of cell loss is severe in the substantia nigra pars compacta, intermediate in the ventral tegmental area and cell group A8, but nonexistent in the central gray substance. This heterogeneity provides a good paradigm for analyzing the factors implicated in this differential vulnerability. So far, the neurons that degenerate have been shown to contain neuromelanin, high amounts of iron, and no calbindin<sub>28K</sub>, and to be poorly protected against oxidative stress. By contrast, the neurons that survive in Parkinson's disease are free of neuromelanin, calbindin<sub>D28</sub>-positive, contain low amounts of iron, and are better protected against oxidative stress. The analysis of the pattern of cell loss in Parkinson's disease may thus bring new clues as to the mechanism of nerve cell death in Parkinson's disease.

Index Entries: Substantia nigra; oxidative stress; calcium; nerve cell death; pathophysiology.

## What Can We Learn from the Pattern of Cell Loss in Parkinson's Disease?

Parkinson's disease represents a good paradigm for studying the pathophysiology of neurodegenerative disorders because the appearance of the clinical symptoms (akinesia, rigidity, and tremor) is linked to the loss of dopaminergic neurons in the brain of the patients (Fig. 1). Yet, degeneration of dopaminergic neurons is heterogeneous in Parkinson's disease (1). Indeed, the degree of cell loss varies from one catecholaminergic cell group to another in the mesencephalon of the patients: It is severe in the substantia nigra pars compacta (76% loss), intermediate in the substantia nigra pars lateralis (34% cell

loss), ventral tegmental area (55% cell loss), and catecholaminergic cell group A8 (31% loss), and almost nonexistent in the central gray substance (7% loss). From this differential vulnerability of dopaminergic neurons in Parkinson's disease, we can conclude that it is not solely the dopaminergic phenotype that makes the neurons susceptible to the causative agent of the disease. The data suggest rather that some other factors (deleterious or neuroprotective) make some dopaminergic neurons, such as those located in the substantia nigra pars compacta, susceptible to degeneration, and afford effective protection against nerve cell death to others, such as those located in the central gray substance. An analysis of these parameters may thus provide fresh clues concerning the mechanism of nerve cell death in Parkinson's disease. The following paragraphs

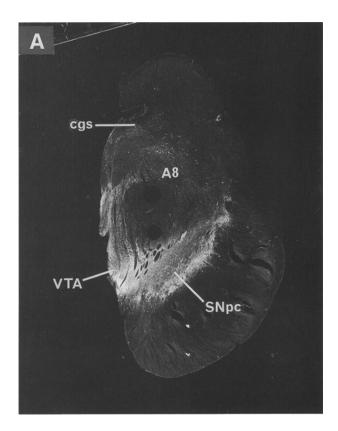




Fig. 1. Reverse contrast photograph of tyrosine hydroxylase immunostained sections from the mesencephalon of a normal human control (A) and a patient with idiopathic Parkinson's disease (B). Note that in the parkinsonian brain, only a few neurons survive in the substantia nigra (star). A8: catecholaminergic cell group A8, cgs: central gray substance, CP: cerebral peduncle, RN: red nucleus, SN: substantia nigra. Bar indicates 2 mm.

shall review some of the factors that may influence the vulnerability of dopaminergic neurons in Parkinson's disease.

# Is Oxidative Stress Involved in the Differential Vulnerability of Dopaminergic Neurons in Parkinson's Disease?

Because Parkinson's disease is characterized by the loss of dopaminergic neurons and dopamine catabolism results in the production of oxygen-free radicals, a link between oxidative stress and the mechanism of nerve cell death has long been suspected in Parkinson's disease. Indeed, an increased lipid peroxidation (which may represent the consequence of oxidative stress or also be the consequence of nerve cell death) has been reported in the substantia nigra of patients with Parkinson's disease, suggesting an

involvement of free radicals in the mechanism of nerve cell death (2). Yet, as previously mentioned, not all catecholaminergic neurons degenerate in Parkinson's disease, raising the possibility that the degree of oxidative stress varies from one catecholaminergic cell group to another. In line with this concept, it should be noted that the metabolism of dopamine is not identical in all catecholaminergic neurons in humans. Indeed, in primates, dopamine is transformed into a pigment called neuromelanin, by an auto-oxidation reaction that produces oxygen-free radicals (3). Thus, one may assume that the amount of free radicals produced is higher in melanized than in nonmelanized neurons. Interestingly, a link between the presence of neuromelanin in dopaminergic neurons and their vulnerability in Parkinson's disease has been reported (1). These data suggest that, in Parkinson's disease, the neurons in which the production of oxygen-free radicals is the highest are more susceptible to degeneration.

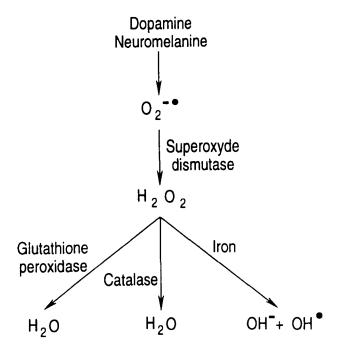


Fig. 2. Schematic representation of oxygen-free radicals metabolism.

Other data suggest that the presence of neuromelanin is not the only factor involved in the mechanism of nerve cell death in Parkinson's disease: (1) Some heavily melanized neurons still survive in the substantia nigra of patients with this disease, and (2) only a slight difference in the degree of melanization of dopaminergic neurons is observed in the substantia nigra between control and parkinsonian patients (4,5). These results suggest that other factors are associated with the presence of neuromelanin during the degenerative process of dopaminergic neurons in Parkinson's disease and that they may influence the distribution of the lesions.

The abundance of oxygen-free radicals in the vulnerable catecholaminergic regions of the mesencephalon depends not only on their production, but also on their rate of degradation. Free radicals scavenging enzymes may thus play a major role in oxidative stress. The first enzyme involved in free radical defense mechanisms is superoxide dismutase, which catalyzes the transformation of superoxide radicals into hydrogen peroxide (Fig. 2). Two types of superoxide dismutases have been described in the human brain: a soluble cytosolic copper/zinc-dependent enzyme and a particulate manganese-dependent enzyme located in the mitochondria. Interestingly, both enzymes have been shown to be particularly concentrated in the mela-

nized neurons of the substantia nigra. Indeed, the messenger RNA for the copper/zinc-dependent enzyme has been located almost exclusively in the melanized neurons in the substantia nigra (6). More recently, manganese-dependent superoxide dismutase was also shown to be present in the melanized neurons of the substantia nigra (Fig. 3) (7).

The presence of these two enzymes in the melanized neurons of the substantia nigra suggests that they might need a high level of these enzymes to protect themselves against oxidative stress. In this context, the biochemical measurements of cytosolic or particulate superoxide dismutase activity reported in the substantia nigra in Parkinson's disease indicate that under pathological circumstances, the system may compensate for, or even correct, an increase in the production of superoxide anions (8,9). Nevertheless, the exact role played by superoxide dismutase in the pathophysiology of Parkinson's disease is not yet clear. Indeed, mice endowed by genetic manipulations with additional human copper/zinc-dependent superoxide dismutase gene are resistant to 1-methyl-4-phelyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication, suggesting that increased expression of superoxide dismutase may protect dopaminergic neurons against nerve cell death (10). By contrast, high levels of superoxide dismutase observed in the substantia nigra may have deleterious consequences for the dopaminergic neurons. Indeed, the reaction product of superoxide dismutase is hydrogen peroxide (Fig. 2), which is responsible for the production of highly toxic hydroxy radicals. Superoxide dismutase may thus have some protective effects by scavenging superoxide radicals, but may also have some deleterious properties if the hydrogen peroxide produced is not detoxified.

Two enzymes are involved in hydrogen peroxide detoxification: catalase and glutathione peroxidase (Fig. 2). Of these enzymes, glutathione peroxidase, which catalyzes the transformation of hydrogen peroxide into water, is interesting because it is heterogeneously distributed in the human midbrain: (1) Glutathione peroxidase is only detected in glial cells and not in the neurons (11), and (2) the density of glutathione peroxidase-containing glial cells is higher in the catecholaminergic cell groups, which are preserved in Parkinson's disease, than in those in which cell loss is severe (11). Taken together, these data on superoxide dismutase and glutathione peroxidase suggest that some catecholaminergic neurons are better protected against

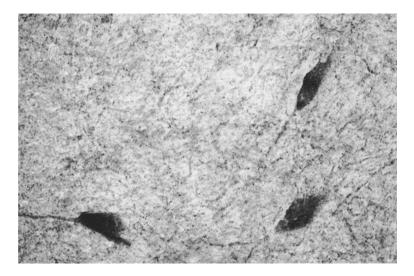


Fig. 3. Manganese-dependent superoxide dismutase immunoreactive cells in the substantia nigra. Note that strong immunostaining is detected in the large melanized neurons.

oxidative stress by free radical scavenging enzymes than others and that this may in turn participate in the selective vulnerability of some of them in Parkinson's disease.

Yet, if the rate of production and of degradation of free radicals analyzed in the normal human mesencephalon might explain why some neurons are more sensitive than others to oxidative stress, it does not explain why oxidative stress perhaps occurs in Parkinson's disease. If one wants to give an explanation for the fact that the increased lipid peroxidation observed in the substantia nigra in Parkinson's disease perhaps represents the consequence of oxidative stress, one has to assume that some factors that induce oxidative stress are present in increased concentration in Parkinson's disease. Of the different hypotheses, the involvement of iron is supported by several lines of evidence. First, iron is a substance capable of dramatically increasing the production of hydroxy radicals from superoxide radicals. Second, several groups of investigators have reported an increased iron content in the substantia nigra in Parkinson's disease (12–18). Last, the increased iron content observed in the substantia nigra in Parkinson's disease does not seem solely to represent the consequence of iron sequestration in glial cells resulting from the gliosis observed in this structure (11). Indeed, iron levels measured using X-ray microanalysis (15,18) or LAMA techniques (16) have been shown to be increased in the melanized neurons of the substantia nigra. Yet, the results are difficult to interpret since biochemical abnormalities observed in the brain of patients with Parkinson's disease, long after the onset of the disease, may be associated with the mechanism of nerve cell death or simply represent a consequence of cell degeneration. Nevertheless, the former hypothesis is the more likely given the fact that (1) iron levels in Parkinson's disease are normal in the central gray substance, where catecholaminergic neurons do not degenerate, and (2) in progressive supranuclear palsy, another parkinsonian syndrome associated with the loss of dopaminergic neurons in the substantia nigra, iron levels are normal in the substantia nigra (15). Taken together, the data suggest that the increased iron content in the substantia nigra may participate in the mechanism of nerve cell death by inducing the production of oxygen-free radicals in a cerebral region particularly vulnerable to oxidative stress, as indicated above. Moreover, the fact that iron binds to neuromelanin may provoke its progressive release in the structure and progressively poison dopaminergic neurons (19) by generating oxygenfree radicals.

### Do Other Factors Influence the Vulnerability of Dopaminergic Neurons in Parkinson's Disease?

In addition to the generation of oxygen-free radicals, several other factors may influence the vulnerability of dopaminergic neurons in Parkinson's

Table 1
Effect of Fibroblast Growth Factor, Brain-Derived Neurotrophic Factor, and Epidermal Growth Factor on Dopaminergic Cells\*

Fibroblast growth factor

Increases growth and survival of catecholaminergic cells

Stimulates uptake of dopamine

Prevents the neurotoxic effect of MPTP

Brain-derived neurotrophic factor

Prevents neuronal death in vivo

Enhances the survival of dopaminergic neurons in mesencephalic cell cultures

Protects cultured nigral dopaminergic neurons from the neurotoxic effect of MPP+

Epidermal growth factor

Stimulates dopaminergic development in vitro

Increases tyrosine hydroxylase activity and mRNA levels

Protects midbrain dopaminergic neurons against MPTP intoxication

Data have been taken from refs. 30-45.

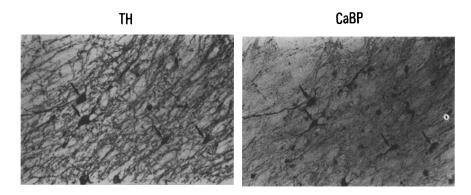


Fig. 4. Photomicrographs taken from sections of the mesencephalon from a control brain stained successively for calbindin  $D_{28}$  and tyrosine hydroxylase.

disease. Of these, compounds capable of promoting cell survival or growth are of particular interest. Neurotrophic factors are necessary for cell survival and outgrowth (20). Some neurotrophic factors are active on dopaminergic neurons (Table 1). Moreover, as shown for brain-derived neurotrophic factor (BDNF), these compounds may even protect the dopaminergic neurons against MPP+ in vitro (21). This suggests that a defect in the production of BDNF or a BDNF-like factor, or their lack of action may participate in the pathophysiology of Parkinson's disease. Nevertheless, such abnormalities have not yet been reported in the substantia nigra in Parkinson's disease. Similarly, for epidermal growth factor (EGF), another neurotrophic factor acting on dopaminergic neurons, the density of its receptors is apparently unchanged in the substantia nigra of patients with Parkinson's disease (22). Although these compounds do not seem to participate in nerve cell death in Parkinson's disease, they may be of potential interest, since they could become an interesting therapeutic tool capable of promoting cell survival and sprouting.

Another factor that may be involved in the death of dopaminergic neurons in Parkinson's disease is a rise in intracellular calcium concentrations. Indeed, abnormal intracellular calcium concentrations can lead to mitochondrial dysfunctions, cytosqueletal alterations, activation of proteases, and endonucleases finally inducing nerve cell death (23–25). Accordingly, it has been proposed that calcium-binding proteins, thought to maintain intracellular calcium homeostasis, have a neuroprotective function by acting as an intracellular buffer (26). Calbindin D<sub>28K</sub> (calbindin) is one of these proteins and is of particular interest because it is detectable in some catecholaminergic neurons in the midbrain (27) (Fig. 4). Yet, not all catechola-

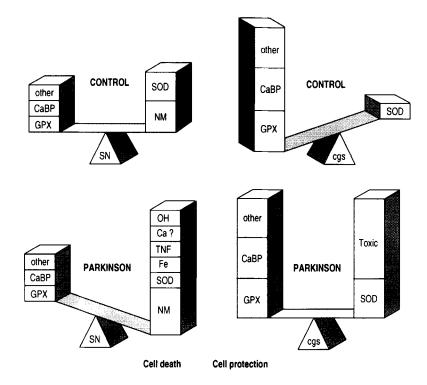


Fig. 5. Schematic representation of the equilibrium between neuroprotective or neurotoxic factors in the substantia nigra (SN) and the central gray substance (cgs). In the central gray substance, the neuroprotective compounds (GPX, CaBP, and so forth) are particularly abundant, whereas in the substantia nigra, the amount of neurotoxic (SOD, NM, and so on) and neuroprotective compounds may only just be in equilibrium. Under pathological circumstances, the amount of toxic compounds, such as iron, calcium, or other molecules, may increase and lead to nerve cell death in the substantia nigra, but not in the central gray substance.

minergic neurons are calbindin positive. The expression of calbindin varies systematically from one catecholaminergic cell group to another. The percentage of calbindin-positive neurons is high in the central gray substance, low in the substantia nigra pars compacta, and intermediate in catecholaminergic cell group A8 and in the ventral tegmental area. In Parkinson's disease, there is a significant negative correlation between the percentage of calbindin-positive tyrosine hydroxylase-positive neurons in the different cell groups and the loss of neurons within them (27). This suggests that the presence of calbindin in some of the catecholaminergic neurons may protect them against the rise in calcium concentrations, which is thought to represent the final effector of nerve cell death. This concept is further supported by the fact that the catecholaminergic neurons containing calbindin are also less vulnerable to MPTP intoxication (28,29). The absence of calbindin in some catecholaminergic neurons may thus facilitate their entry into a calcium-mediated process leading to nerve cell death.

### Conclusion: Catecholaminergic Neurons Are Differentially Vulnerable to Nerve Cell Death in Parkinson's Disease

The above data suggest that two populations of dopaminergic neurons may exist in the mesencephalon. One population is represented by neurons located in the central gray substance, catecholaminergic cell group A8, and the ventral tegmental area, and the other located in the substantia nigra pars compacta, the ventral tegmental area, and catecholaminergic cell group A8. These two populations of neurons are differentially vulnerable to nerve cell death. This differential vulnerability may result from a different equilibrium between neurotoxic and neuroprotective factors between the two types of neurons (Fig. 5). In the central gray substance, the neuroprotective compounds are particularly abundant, whereas in the substantia nigra, the amount of neurotoxic and neuroprotective compounds may only just be in equilibrium. Under pathological circumstances, the amount of toxic compounds, such as iron or other molecules, may increase. Given the abundance of neuroprotective substances in the central gray substance, the catecholaminergic neurons may be spared, whereas in the substantia nigra, an excess of neurotoxic compounds may lead to nerve cell death. Accordingly, because nigral neurons are particularly vulnerable, different types of injuries may lead to nerve cell death in this structure. Such a concept is all the more likely given the different possible etiologies of parkinsonian syndromes. Indeed, it seems reasonable to assume that the agent that causes nerve cell death in idiopathic, familial, toxic, and so forth, parkinsonism is different. Yet, the pattern of cell loss in the different catecholaminergic cell groups of the mesencephalon is very similar in all these disorders, suggesting that intrinsic properties of some catecholaminergic neurons make some of them particularly vulnerable to different kinds of stress.

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