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Pathogenesis of Parkinson's Disease

Prospects of Neuroprotective and Restorative Therapies

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Abstract

Parkinson's disease (PD) is caused by the degeneration of dopaminergic neurons of substantia nigra projecting to striatum. The cause of idiopathic PD is obscure, and most cases are sporadic. It is widely accepted that there is a genetic component of the disease, and the earlier the age of onset, the greater the likelihood that genetic factors play a dominant role. Oxidative stress of the substantia nigra seems to contain the driving force for neurodegeneration, leading to a destructive "toxic cycle." The most prevalent therapy is levodopa administration, but it is not efficacious after several years of treatment. Several alternative therapies are currently being explored, such as neuroprotective approaches. Compounds with potentially neuroprotective efficacy such as selegiline, dopamine agonists, riluzole, creatine, and coenzyme Q10 are currently being tested. Trophic factors represent another class of neuroprotective compounds, but their intracerebral administration is difficult to achieve. In this respect, a potentially useful therapeutic approach is grafting cell vectors that release trophic molecules that stimulate regeneration in the damaged nigrostriatal system. Promising results have been obtained with fibroblasts engineered to secrete glial cell line-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) or viral vectors expressing GDNF. We have tested the suitability of intrastriatal grafts of chromaffin cells obtained from the Zuckerkandl's organ, which exert beneficial effects in parkinsonian rats, and release trophic factors such as GDNF and transforming growth factor-β_1 (TGF-β_1).

Index Entries: 6-OHDA; Parkinson disease; GDNF; TGF-β_1; trophic factors.

Introduction

Parkinson's disease (PD) is caused by the loss of dopaminergic neurons of substantia nigra projecting to striatum, leading to dopamine deficit in the basal ganglia. The disease is characterized by serious functional disturbances such as tremor, rigidity, akinesia, and slowness of spontaneous movements. PD is one of the major neurodegenerative disorders, and was originally described by James

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Parkinson in 1817. His remarkably complete account gives this definition: "Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with propensity to bend the trunk forward, and to pass from a walking to a running pace, the senses and intellects being uninjured." The disease affects mostly people older than 60 yr, where prevalence is around 0.5%. The defining neuropathological feature in PD is the presence of Lewy bodies in the substantia nigra together with nigral cell loss (higher than 80% once motor deficits appear). The Lewy bodies, described by this author in 1912, are inclusions (5–25 μm in diameter) with a dense eosinophilic core with a pale surrounding halo. The cause of disease is unknown, even though it is widely accepted that there is a genetic component. Thus, twin studies have revealed that there is a high concordance rate for monozygotic twins (55%) that is three times higher than that for dizygotic twins (18%), implying a genetic contribution *(1).* However, this concordance is only observed in twins with an age of PD onset lower than 50 yr. This fact is important, indicating that environmental cues also play a role. At the present moment, it is thought that there is a complex interplay between genetic and environmental influences in the causation of PD.

Pathogenesis

Genetic and Environmental Factors

The cause of idiopathic PD, responsible for nigral dopaminergic cell loss, is obscure. Most cases of PD are sporadic, and familial cases are rare (the estimated familial incidence is 1–2%). As explained, the earlier the age of onset, the greater the likelihood that genetic factors play a dominant role. Thus, a study revealed that the risk to siblings of an isolated case of PD varies with age of onset *(2).* The risk is 1 in 12 when the age is lower than 45 yr; 1 in 20 between 45 and 55 yr; 1 in 26 for 55 to 65 yr, and 1 in 71 for older than 65 yr. Twin studies have revealed that there is a high concordance rate for monozygotic twins (55%) that is three times higher than that for dizygotic twins (18%), if the age of onset of PD is lower than 50 yr.

Several genes have been linked to the pathogenesis of PD. The first PD gene to be cloned was identified in an Italian-American family. Mean age of onset of disease was 46 yr, and linkage to markers on chromosome 4q21-q23 was demonstrated. This locus was designated PARK1, and it represents a gene encoding for the protein α-synuclein. Later, it was observed that this protein is one of the principal components of Lewy bodies, revealing its importance in the PD pathogenetic process *(3).* Another mutation, located at the locus 6q, was discovered in Japanese patients *(4).* Age of onset was younger, and the gene was found to encode for the protein parkin, a protein with an E3 ubiquitin ligase function. This fact implicates parkin in the ubiquitine–proteasome system *(5),* whose dysfunction seems to be involved in PD as well. Finally, other PARK loci, named PARK6, has been linked to eight European families. The locus is 1p36-35, but the gene is unknown. This PD is similar to idiopathic PD, since age of onset is late and the progression is slow *(6).*

In the abscence of a genetic cause, environmental influences appear to play an important role in the cause of PD. Rural living has been associated with an increased risk for PD *(7).* Rural living is associated with the use of pesticide in agriculture, and pesticides such as dieldrin and dithiocarbamates have been shown to be associated with increased PD *(8,9).* Another clear environmental factor is MPTP, a contaminant of illicit heroin. MPTP was found to cause PD within 7–14 d in heroin addicts *(10).* After MPTP administration, this compound is converted to the toxin MPP+ by monoaminooxidase B, an enzyme abundant in the monoaminergic neuronal system, and this toxin is selectively taken by dopamine neurons, causing their death. For this reason, MPTP is frequently used in many laboratories for inducing PD (even though without the induction of Lewy bodies) in an animal model of the disease

in rodents and primates. It is interesting to note that pesticides and MPP+ are mitochondrial inhibitors, and it has been detected a deficiency of mitochondrial complex I in the substantia nigra in a high proportion of PD patients *(11).* MPP+ is a specific inhibitor of mitochondrial complex I, leading to increased free radical release and oxidative damage, which seems to be the main cause of dopaminergic neuronal death characteristic of PD.

Is Cell Death in Substantia Nigra Caused by Oxidative Damage?

The generation of reactive oxygen species is part of the normal cellular metabolism. Free radicals are normally scavenged by cells, through conversion of superoxide ions to hydrogen peroxide under the control of superoxide dismutase, and then the formation of water from the reaction of hydrogen superoxide with reduced glutathione (GSH), under the control of glutathione peroxidase. Oxidative stress results from insufficient scavenging of reactive oxidative species. Nigral dopaminergic neurons are exposed to higher oxidative stress because the metabolism of dopamine gives rise to dopamine–quinone species, superoxide radicals, and hydrogen peroxide. Hydrogen peroxide is innocuous but it can be converted into cytotoxic hydroxyl radicals, in a reaction catalyzed by iron (Fenton reaction). This reaction can be dangerously enhanced if GSH levels are reduced and there is an excess of iron (Fe^{2+}). Superoxide is not a highly reactive molecule but if nitric oxide levels are enhanced, as seems to be the case in PD, it can react with nitric oxide to form peroxynitrite and hydroxyl radicals, strong oxidizing agents *(12).* Nitric oxide can displace iron from ferritin thereby enhancing free iron levels. Figure 1 shows the main reactive oxidative species that contribute to the pathogenesis of PD.

There are several indicators that substantia nigra in PD is subjected to increased oxidative stress, and nigral iron levels have been found to be elevated in PD patients (129%) *(13,14).* Thus, GSH levels are decreased by 40% *(11),* although the role of this decrease has been questioned since these levels are not considered to be dangerous. However, a strong decrease of GSH content has been detected in glia of nigral tissue of PD patients *(15).* The cause of this decrease is not known, but clearly renders cells more sensitive to toxin action and potentiates the toxic effects of glial cell activation. In this context, nitric oxide synthase can be enhanced through glial cell activation *(16),* leading to excess of nitric oxide and the oxidizing agents peroxynitrite and hydroxyl radicals.

The excessive formation of reactive oxygen and nitrogen species in PD increases oxidative damage to proteins, lipids, and DNA. The levels of protein carbonyls—markers of protein oxidation—are twofold higher in the substantia nigra of PD patients *(17).* The levels of lipid hydroperoxides—markers of oxidized lipids—are tenfold higher in the substantia nigra of PD patients *(18).* Finally, 8-hydroxyguanosine—a marker of oxidized RNA and DNA—is also enhanced *(19).* The strong oxidative stress in the substantia nigra can lead to misfolding of proteins such as α-synuclein and parkin. Misfolded α-synucleins (as abnormal β-sheets instead of normal alfa-helices) tend to form protofibrils that precipitate forming fibrils, which in turn constitute the core of the Lewy body. Protofibrils has been shown to be neurotoxic *(20),* pointing to the fact that protofibrils, rather than fibrils, could be the deleterious species. In this context, it was proposed that Lewy bodies could be a protective mechanism, although it has recently been demonstrated that the higher the number of Lewy bodies, the stronger the degree of clinical symptoms, suggesting that Lewy bodies are not protective *(21).* The loss of normal function of α-synuclein could alter the normal vesicle function, since this protein is an important regulator of synaptic vesicle cycle, leading to enhanced intracellular levels of dopamine which are thought to further enhance intracellular oxidative stress as explained *(3).* Furthermore, accumulation of protofibrillar synuclein can saturate the ubiquitin–proteasome pathway (UPP). Normally proteins that are misfolded or damaged are

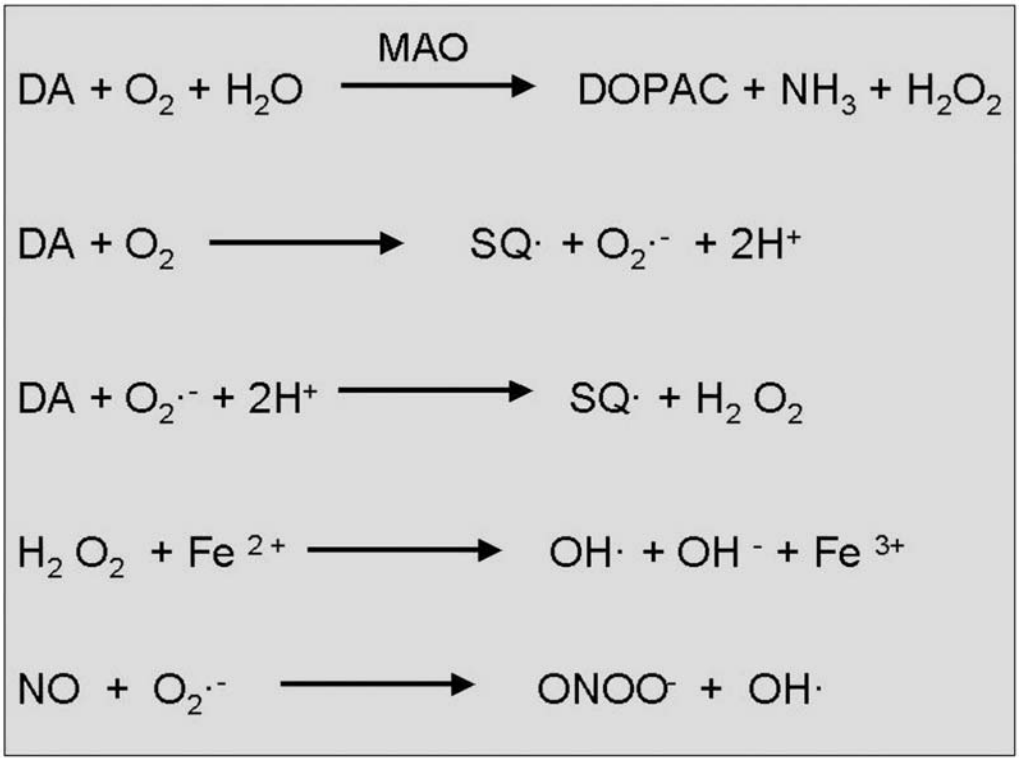

Fig. 1. Reactive oxygen species in Parkinson's disease. Normal metabolism of dopamine leads to DOPAC and hydrogen peroxide under the control of the enzyme MAO, but also to oxidative species such as superoxide anions ($O_2 \cdot^-$), dopamine-quinone species (SQ·), and hydroxyl radicals (OH·). Hydrogen peroxide (H_2O_2) does not usually damage the cells because it is scavenged by glutathione peroxidase, but it can be converted to hydroxyl radicals by the Fenton reaction in presence of reduced levels of glutathione peroxidase and excess levels of Fe^{2+}. Superoxide anions are not normally dangerous because they are converted to hydrogen peroxide under the control of superoxide dismutase. However, if nitric oxide (NO) levels are enhanced, superoxide anions can react with nitric oxide to form peroxynitrite ($ONOO^-$) and hydroxyl radicals, strong oxidizing agents. DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; MAO, monoamine oxidase.

degraded by UPP *(22)*, but there is also evidence that the UPP is impaired in Parkinson's disease *(23)*, not only due to accumulation of misfolded proteins but likely secondary to oxidative stress. A saturated or impaired UPP could lead to a defective handling of misfolding proteins such as α-synuclein and formation of Lewy bodies. Neurodegeneration could account for these oxidative stress phenomena and UPP impairment, although other pathogenic mechanisms have also been proposed, such as mitochondrial dysfunction, excitotoxicity, and inflammation.

Mitochondrial Dysfunction, Excitotoxic Damage, and Inflammation Are Other Causes of Cell Death in the Substantia Nigra Leading to a Destructive "Toxic Cycle"

Complex I activity is decreased in the substantia nigra of PD patients *(11)*, a fact that is specific to PD. However, this decline is moderate and cannot account for the extent of cell death in this nucleus. Iron-mediated free radical production can inhibit complex I activity *(24)*, and it is likely that there is an interaction

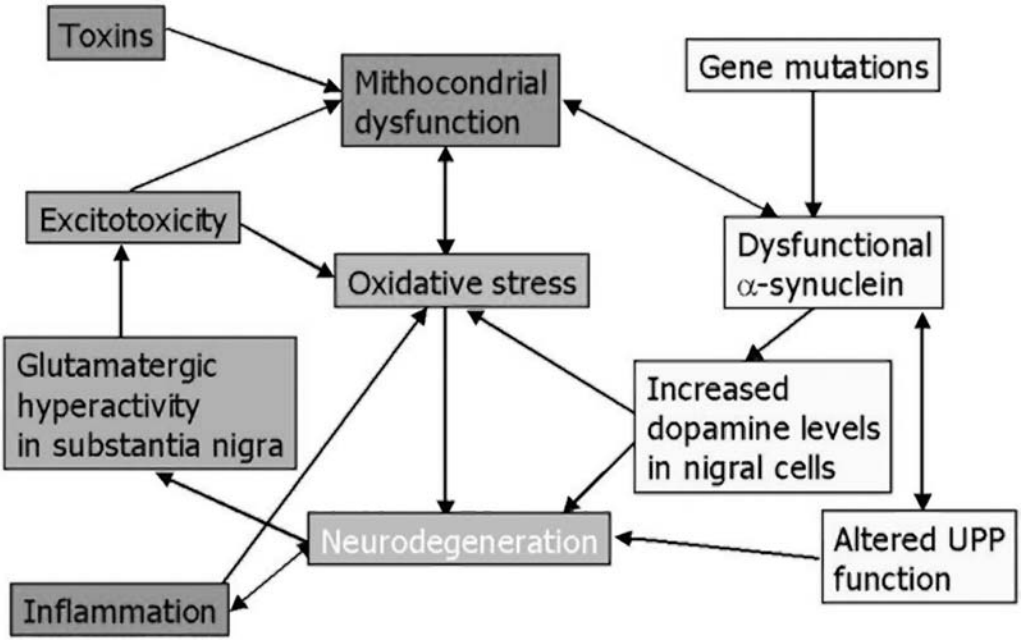

Fig. 2. The destructive toxic cycle: oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammation enhance each other in a cycle of toxicity leading to nigral cell degeneration in Parkinson's disease. UPP, ubiquitin-proteasome pathway.

between oxidative stress and mitochondrial inhibition, enhancing each other in a cycle of toxicity (*see* Fig. 2). Regarding excitoxicity as another factor involved in the pathogenesis of PD, the subthalamic nucleus becomes hyperactive once substantia nigra degeneration is strong, leading to enhanced glutamatergic release within the nigra. Excess of glutamate can damage dopaminergic cells of the substantia nigra because glutamate enhances the permeability to calcium through NMDA receptors located on these neurons. Enhancement of intracellular calcium can induce cell degeneration and this phenomenon may further aggravate the progression of the disease leading to accelerated nigral neurodegeneration. Apart from signs of mitochondrial dysfuntion and excitotoxicity, there are also signs of neuroinflammatory reactions in PD because marked increases in cytokine levels have been found in the striatum and cerebrospinal fluid (CSF) of PD patients, including proinflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 *(25).* There is also upregulation of inflammatory-associated factors such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase. It has been postulated that once the neurodegeneration process begins, T-lymphocytes are somehow activated within the substantia nigra, which in turn induce the activation of glial cells. These glial cells release proinflammatory factors and nitric oxide leading to apoptosis and oxidative stress, respectively *(26).* Hence, neuroinflammation is another amplifying system leading to cell degeneration.

In summary, although oxidative stress seems to contain the driving force behind neurodegeneration (unknown), it is included in a destructive "toxic cycle" characterized by mitochondrial dysfunction, excitotoxicity, and inflammation which enhance each other and ultimately aggravate the degree of neurodegeneration. This fact explains the rapid progression of PD once disabilitites and clinical symptoms emerge.

Therapies

Enhancement of the Dopaminergic Tone

Levodopa Can Enhance the Dopaminergic Tone in the Damaged Striatum

The most prevalent therapy for PD is oral levodopa administration, which is converted into dopamine in the brain by surviving dopamine cells (and likely glia), leading to a remarkable amelioration of the disorder. The use of this highly effective compound followed the demonstration by Hornykiewicz that there is a profound loss of dopamine in the striatum of PD. This compound is usually administered in association with carbidopa in order to reduce peripheral levodopa inactivation (very high in the liver and brain capillaries), and this combination is particularly effective for alleviation of akinesia and rigidity in early- and middle-stage PD. Other drugs can be used in combination such as direct-acting dopamine agonists or anticholinergics.

Unfortunately, levodopa and dopaminergic drugs are not efficacious after several years of treatment, likely because neuronal loss progresses precluding the intracellular conversion of levodopa. Many PD patients develop severe side effects, such as dyskinesias at peak dose and on–off fluctuations in drug effectiveness. For this reason, several alternative therapies are currently being explored, such as new pharmacological tools and cellular substitution.

Transplantation of Dopamine-Secreting Cells as Another Approach for Enhancing Dopaminergic Tone Within the Striatum

It is reasonable to think that since levodopa significantly ameliorate functional deficits in PD, grafting dopamine-secreting cells to provide a constant source of dopamine should be as efficient as systemic levodopa therapy. In this context, intrastriatal grafting of dopamine-secreting cells obtained from neural or chromaffin tissues—such as fetal mesencephalon, adrenal medulla, and carotid body—has been reported to ameliorate functional deficits in animal models of PD *(27–31),* and is considered as a promising treatment for human PD. However, its clinical use is either still restricted to only a few cases, is under discussion, or has been abandoned. Thus, clinical trials have shown that mesencephalic dopamine neurons obtained from human embryo cadavers can survive and function in the brain of patients with PD *(32,33);* long-lasting functional improvement has been reported in many grafted patients. However, major limiting factors regarding fetal mesencephalic cells include the ethical, practical, and safety issues associated with tissue derived from aborted human fetuses, and the difficulty in obtaining sufficient viable embryonic mesencephalic tissue *(34,35).* Furthermore, in the recent Denver/New York study, severely disabling dyskinesias were reported to appear in a significant number of grafted patients, even in the absence of levodopa treatment *(36).* This side-effect seems to be caused by unbalanced partial recovery of the dopaminergic tone in different transplanted areas of both putamina without supernormal levels of FDOPA uptake, as revealed by PET *(37).* This fact further complicates the outcome of neuronal transplantation for parkinsonism. On the other hand, adrenal cells were the first dopamine-secreting cells to be investigated, but these cells are no longer used because their long-term survival is very poor in the brain (even after autotransplants), and beneficial effects are transient, both in Parkinson's patients or animals *(35,38).*

Finally, the clinical effects of glomus cell transplants still have to be investigated. A major advantage of glomus cells, like adrenal ones, is that they can be used for autotransplantation, thereby avoiding tissue rejection and the need for immunosupression therapy. However, their clinical efficacy is poor in advanced PD in humans where the carotid body is known to also be affected by dopaminergic cell-degeneration, and possible side-effects such as dyskinesias appearing, as with other dopamine-secreting cells, due to unbalanced focalized increases of striatal dopamine.

Neuroprotection and Neurorestoration

Neuroprotection Can Be Attained With Pharmacological Tools

As explained, neurodegeneration in PD is associated with a cascade of events or a "toxic cycle," including oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammation *(39);* and several theoretical neuroprotective strategies can be defined. However, since the driving force that initiates neurodegeneration is not known, the neuroprotective therapy should block the neurodegenerative process to be effective. Several compounds with potential neuroprotective efficacy have been tested. Thus, since MPTP-induced parkinsonism in monkeys were prevented by deprenyl (selegiline, 40), a clinical double-blind study was carried out using this compound. This so-called DATATOP study revealed that deprenyl can delay the emergence of disability treatment with levodopa. In this study, 800 patients were treated with deprenyl (10 mg/d) and tocopherol (2000 IU), or tocopherol alone (placebo group) in a double-blind study. After 12 mo, levodopa was required in 26% of selegiline recipients compared with 47% of those belonging to the control group. Deprenyl is an inhibitor of MAO-B, but it seems to act as an antiapoptotic drug, most likely due to its metabolite desmethyl-deprenyl. However, selegiline does not stop the pregression of the disease *(41),* and after many years of treatment those patients receiving selegiline are no less impaired than those who have not received the drug *(42–44).* Regarding other compounds with potential therapeutic efficacy, dopamine agonists exert a direct antioxidant effect and they also scavenge hydroxyl radicals, as demonstrated by several authors *(45,46).* The dopamine agonists bromocriptine, pergolide, and pramipexole through their action as antioxidants and free radical scavengers, might be neuroprotective as well *(47,48).* For instance, pramipexole protects substantia nigra from degeneration in MPTP-treated marmosets *(48).* Two independent studies (the CALM-PD and REAL-PET studies) have shown that initial treatment of PD patients with the dopamine agonists pramipexole or ropinirole seem to reduce the decline of nigrostriatal function, as revealed by ^{15}flurodopa uptake on PET in comparison with patients receiving only levodopa *(49).* However, these studies are not conclusive and additional work is required.

Another pharmacological approach is trying to slow down the overactivity of glutamatergic neurons particularly at the level of the subthalamic nucleus. Riluzole is an antiglutamatergic agent that acts by inhibiting sodium channels and prevents the release of glutamate. This drug protects dopamine neurons in animal models of the disease *(50,51).* There are two multicenter placebo-controlled trials currently in course, but the results have not been yet published. Finally, mitochondrial dysfunction plays a role in the pathogenesis of PD, as explained, and bioenergetics agents such as creatine, coenzyme Q10, or nicotinamide may be neuroprotective in PD. Creatine and coenzyme Q10 have been shown to protect dopamine neurons in MPTP-treated rodents *(52,53).* A clinical trial has revealed that there is a dose-dependent reduction in disease which correlates well with plasma levels of coenzyme Q10 *(54).* This drug is currently used by some neurologists trying to slow PD progression in early-stage patients.

Trophic factors represent another class of neuroprotective compounds that could be used as pharmacological tools. Some trophic factors have demonstrated their potential to be potent neuroprotective agents for specific populations of neurons. Concretely, dopaminergic neurons are sensitive to a variety of trophic factors. Thus, members of the fibroblast growth factor family (FGF-1, FGF2), TGF-α, IGF-1, BDNF, and neurotrophin 4 exert neurotrophic influences for dopaminergic nigrostriatal neurons in vitro and in vivo *(55).* The most potent trophic factor for dopaminergic striatal neuron is GDNF, which belongs to the TGF-β superfamily. In animal models of PD, intraventricular injection of GDNF induces a long-term increase in striatal dopamine *(56,57).*

Table 1
Potentially Relevant Pharmacological Tools With Anti-Parkinsonian Neuroprotective Capacity

Class	Compound	Mechanism of action
Inhibitor of MAO-B	Deprenyl	Antiapoptotic
Dopamine receptor agonists	Bromocriptine Pergolide Prampexole	Antioxidants and scavengers of hydroxyl radicals
Antiglutamatergic	Riluzole	Reduction of glutamate excitotoxicity through prevention of glutamatergic release onto substantia nigra
Bioenergetics	Creatine Coenzyme Q10 Nicotinamide	Amelioration of mitochondrial dysfunction
Trophic factors	GDNF FGF-1, FGF-2 BDNF IGF-1 TGF-α	Slowing down dopaminergic cell death and regeneration of damaged circuit through sprouting of surviving dopaminergic neurons

However, clinical trials using intraventricular injections of GDNF failed to improve functional deficits in PD patients and nigrostriatal function was not augmented *(58).* Furthermore, some side effects, such as cephalea and Lhermitte's signs, were experienced by some patients. Thus, intraventricular GDNF in humans appears to be the wrong delivery method *(59).* A study was performed for advanced PD patients in which GDNF was directly injected into the striatum *(60).* However, neurotrophic effects of GDNF were focalized to the site of injection, and side-effects, such as Lhermitte's sign, were reported, likely caused by meningeal irritation. Table 1 shows a summary of potentially relevant pharmacological tools with neuroprotective capacity.

Transplantation of Neuroprotective Vectors Can Help Restaurate the Damaged Nigrostriatal Circuit

Another strategy is to introduce potentially neuroprotective molecules to prevent cell death, slow down the process, or even stimulate regeneration in the damaged dopaminergic nigrostriatal system by using cell or viral vectors. These vectors exert a "dopaminotrophic" action and when grafted cell vectors give rise to neuritic processes extending far away from the site of implantation, the trophic action is widespread and more effective *(34).* This is quite a different approach to grafting dopamine-secreting cells, since a more extense reinnervation and tissue regeneration is searched rather than focalized enhancement of dopamine release. As explained in the previous section, GDNF and BDNF have potent in vivo effects *(61–64).* Apart from being potential pharmacological tools, these factors have also been administered to the brain by using cell or viral vectors. Thus, promising results have been obtained with fibroblasts engineered to secrete GDNF *(65)* or BDNF *(66,67)* and with viral vectors expressing GDNF *(68,69).* In vivo neuroprotective effects have been reported to be in the range of 40–70% effective in the rescue of nigral dopamine cells. Transforming growth factor-β_1 (TGF-β_1) is another trophic factor that has also been reported to protect dopamine cells in vitro *(70),* and to act as a cofactor that potentiates the neurotrophic actions of GDNF in vitro and in vivo *(71,72).* In this context, the anti-Parkinsonian effects of cell grafts of the chromaffin lineage are attributed not only to dopamine release

from grafted cells, but also to dopaminergic reinnervation of the denervated striatum *(28,30,31, 34,73);* chromaffin cells are also known to express and release GDNF and TGF-β_1 *(74–76).* Grafted adrenal cells seem to work as dopaminotrophic cells that induce sprouting of surviving dopamine nigral cell and the reinnervation of damaged striatum. Recently, grafted dopaminergic carotid body cells have been observed to exert an added neurorestorative effect that has been related to the trophic action of GDNF, because carotid body dopaminergic cells express this trophic factor *(77).*

Extra-Adrenal Cells As Possible "Dopaminotrophic" Vectors for Grafting

Considering the potential neurorestorative properties of chromaffin cells, the author has worked for the first time with other cells of the chromaffin lineage, specifically those belonging to the extra-adrenal tissue of abdominal paraganglia. Among these paraganglia, the Zuckerkandl's organ is the largest extra-adrenal paraganglion *(78)* which is located adjacent to the abdominal aorta in mammals and can easily be removed *(79).* The author has shown that intrastriatal transplantation of extra-adrenal chromaffin cells of the Zuckerkandl's organ induce progressive improvement of functional deficits in parkinsonian rats, and also that grafted cells present a long survival after transplantation *(80).*

There is usually one Zuckerkandl's paraganglion in rats, although two or more accessory smaller paraganglia can be found. From a physiological point of view, the organ is known to represent a secondary source to adrenal medulla of blood noradrenaline *(78).* Histologically, it can be observed that the paraganglion is composed of mesenchyma and chromaffin cells *(79).* Chromaffin tissue inside the organ (~22% of the organ volume) forms fascicles along the longitudinal axis, with the appearance of rounded "cell nests" on coronal sections. As typical chromaffin cells, those of the Zuckerkandl's organ react with potassium dichromate (classical Orth's reaction), and the presence of chromogranin A—a protein that participates on exocytosis and is highly abundant in the chromaffin cell lineage—can be detected by immunohistochemistry. Chromaffin cells possess a typically rounded morphology with diameter ranging from 15 to 20 micrometers. These cells also express tyrosine-hydroxylase (TH), the dopamine synthesizing enzyme, and dopamine-β-hydroxylase (DBH), the noradrenaline synthesizing enzyme. Other authors have reported no expression of phenylethanolamine-*N*-methyl-transferase (PNMT), the adrenaline synthesizing enzyme, in the chromaffin cells of the Zuckerkandl's organ *(81).* Furthermore, these extra-adrenal chromaffin cells express GDNF *(81)*. In conclusion, extra-adrenal chromaffin cells of the Zuckerkandl's organ are noradrenergics and express dopaminotrophic GDNF.

Studies in the author's laboratory have documented a trophic regeneration in parkinsonian rats after transplantation of chromaffin cells of the Zuckerkandl's organ. The potential antiparkinsonian efficacy of Zuckerkandl's organ grafts was tested in hemiparkinsonian rats after unilateral dopamine depletion of the substantia nigra. This animal model parallels the human disorder especially well, and has been extensively employed to monitor the possible antiparkinsonian effects of neural and paraneural grafts, neuroprotective treatments, and new drugs. An overt hemiparkinsonian syndrome is induced after unilateral dopamine depletion, which is characterised by drug-induced asymmetries, sensorimotor neglect, akinesia, and forepaw use asymmetry *(81–83).* Rats were rendered hemiparkinsonian by injecting 6-hydroxydopamine into the left substantia nigra, a toxin which destroys dopaminergic neurons (*see* Fig. 3E). Those animals showing a strong ipsilateral rotational behavior after the administration of amphetamine (>420 turns per hour), indicative of the destruction of more than 85% of the dopaminergic neurons in the substantia nigra *(81),* were selected for grafting. Hemiparkinsonian rats were grafted with aggregates of extra-adrenal cells aimed at the dorsal striatum.

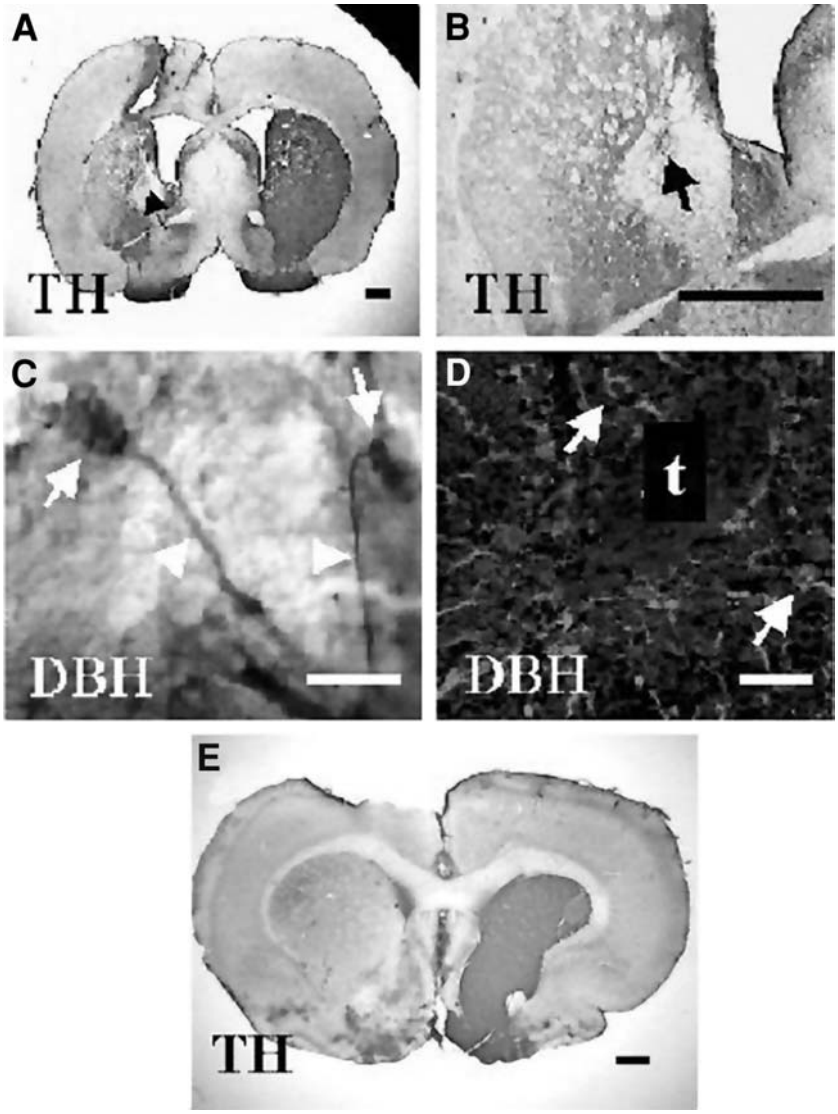

Fig. 3. Morphological features of 5-mo-old transplants of the Zuckerkandl's organ in parkinsonian rats. **(A,B)** Coronal sections of the striatum grafted with extra-adrenal cells of the Zuckerkandl's organ where the TH positive transplant *(arrow)* and the surrounding TH+ reinnervation in the host striatum can be clearly observed. **(C)** Higher magnification of the same transplant labelled for DBH where DBH+ chromaffin cells *(arrows)* with very long neuritic processes *(arrowheads)* are observed. **(D)** Confocal fluorescent micrograph of the graft revealing the presence of DBH+ cells *(arrows)* and many neuritic processes running out of the graft. **(E)** Coronal section of the striatum of a sham parkinsonian rats, where TH positivity *(dark signal)* in the left striatum is nearly absent (compare with contralateral striatum and grafted striatum in A and B). t, transplant. Scale bars: 1mm in A,B,E; 100 µm in C; 50 µm in D. Modified from Fig. 3 (*The Journal of Neuroscience* 21, 9888–9895, 2001, copyright by the Society of Neuroscience).

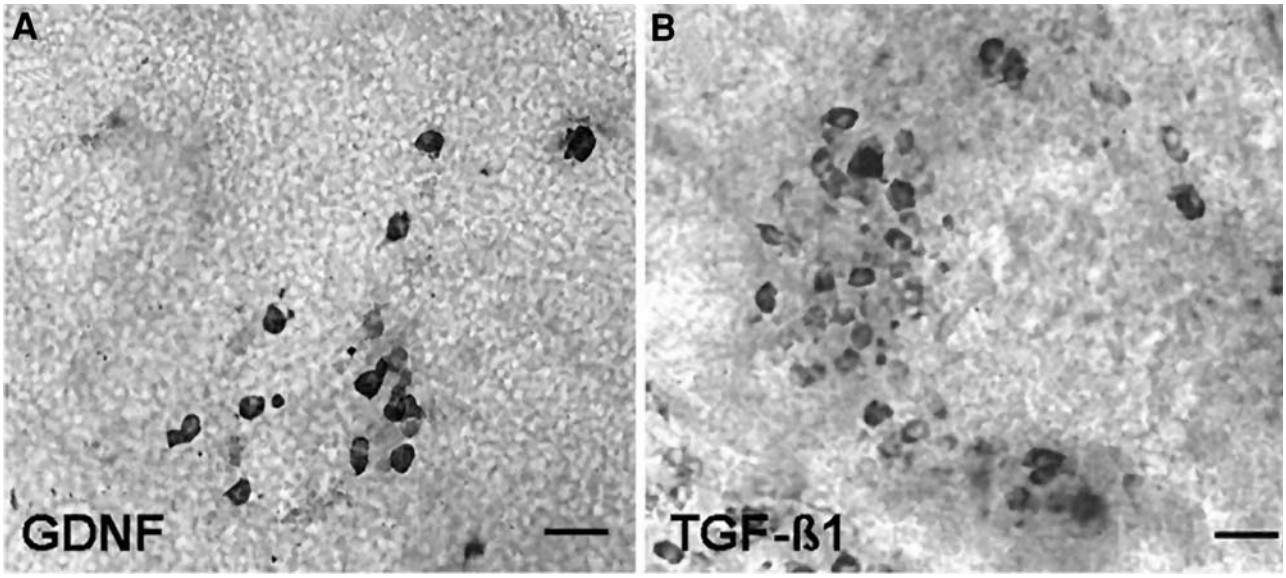

Fig. 4. **(A)** Immunohistochemistry for GDNF and TGF-β_1 in transplants of the Zuckerkandl's organ at 5 mo after grafting, showing the presence of GDNF-positive and **(B)** TGF-β_1-positive chromaffin cells. Scale bars: 50 µm. This figure was published in *The Journal of Neuroscience* 21, 9888–9895, 2001 (copyright by the Society of Neuroscience).

Grafted rats showed a clear behavioral improvement up to 5 mo, as manifested by a progressive and sustained reduction of sensorimotor deficits, forepaw use asymmetry, and amphetamine-induced turning. These functional effects were related to the survival of 13% of grafted cells at 1 and 5 mo after grafting—a remarkable finding that indicates those grafted chromaffin cells surviving the grafting trauma then survived for a long period of time, making them suitable for transplantation. Immunohistochemistry staining revealed the presence of TH+ and DBH+ chromaffin cells inside grafts, many of them with long neuritic processes emerging from the soma (Fig. 3C–D). Besides significant enhancement of TH+ density in the host striatum (indicative of dopaminergic reinnervation; Fig. 3A,B), a reliable increase in the intrastriatal dopamine content was also detected (49% vs normal). This latter fact surely led to behavioral improvements in parkinsonian rats, which are dependent on the recovery of the dopaminergic tone of dorsal striatum *(84–86).* The increase of striatal dopaminergic content after grafting cannot be explained by dopamine cell-release because grafted cells were not dopaminergics (as explained), and it is known that only minute amounts of dopamine can be released from noradrenergic adrenal and extra-adrenal chromaffin cells, as reported by other authors *(87–89).* These findings indicate that the improved dopaminergic tone after grafting can be better accounted for by striatal regeneration due to sprouting of spared dopaminergic fibers. This is consistent with the gradual time-course of functional recovery.

The immunohistochemical study of grafts revealed that grafted chromaffin cells expressed GDNF and TGF-β_1 (*see* Fig. 4), and significant levels of these neurotrophic factors were detected in the striatal tissue, pointing to a chronic release of the factors after transplantation in vivo. GDNF and TGF-β_1 are known to protect dopaminergic neurons from degeneration in vitro and animal models of PD when delivered by injection via transplanted cells or viruses. Hence, the well known neurorestorative action of GDNF and TGF-β_1 could account

for the striatal reinnervation of the host tissue. Clearly, the main advantage of grafts of the Zuckerkandl's organ appears to be the long survival of extra-adrenal chromaffin cells, which allow them to exert a chronic trophic action based on the delivery of GDNF and TGF-β_1, and likely other neuroprotective agents such as neuropeptides and cytokines that are known to be released by chromaffin cells as well *(90).* These findings strongly enhance the potential therapeutic value of the grafts of the Zuckerkandl's organ. Since more available and less controversial alternative sources for antiparkinsonian therapy need to be developed *(91),* this work should stimulate research on the clinical applicability of transplants of the Zuckerkandl's organ in PD, ever considering that the authors have dealt with an animal model of parkinsonism rather than the actual human disorder. A potential advantage in humans is that there are usually two Zuckerkandl's paraganglia, allowing the surgical resection of one of them for use as an autotransplant, thereby avoiding tissue rejection and the need for immunosuppression therapy. For the same reason, the surgical resection of one organ out of two will not have significant physiological side effects.

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