Neurodegenerative Pathways in Parkinson’s Disease: Therapeutical Strategies

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Abstract: Parkinson’s disease (PD), considered one of the major neurological disorders, is characterized by the loss of dopaminergic neurons in the pars compacta of the substantia nigra and by the presence of intraneuronal cytoplasmic inclusions called Lewy bodies. The causes for degeneration of PD neurons remain unclear, however, recent findings contributed to clarify this issue. This review will discuss the current understanding of the mechanisms underlying Parkinson’s disease pathogenesis, focusing on the current and potential therapeutic strategies for human treatment.

Keywords: α-synuclein, neuronal circuits, oxidative stress, mitochondria, proteasome, neuroinflammation, cell death, therapeutics.

ETIOPATHOGENESIS OF PARKINSON’S DISEASE

Parkinson’s disease (PD) is the most common progressive neurodegenerative movement disorder with a prevalence of about 2% in individuals older than 65 years of age. However, the disease can also manifest earlier in life, before 40 to 50 years, referred as early onset PD [1]. It is characterized clinically by tremor, rigidity, bradykiniesia and postural instability, and non-motor symptoms such as cognitive and mood impairment, and autonomic dysfunction can also be observed. Pathologically, PD is characterized by selective neuronal loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), which leads to a drastic depletion of dopamine (DA) levels in the striatum, to which these neurons project [2]. Although the course of the disease is unknown, the leading hypothesis for the death of specific groups of neurons establishes that alterations in protein aggregation, proteasomal system impairment, mitochondrial dysfunction and oxidative stress are the major events that act synergistically causing this devastating disease.

Neuropathological Hallmarks in PD

The most prominent histopathological hallmarks of PD are the intracellular accumulation of insoluble fibrous material, named Lewy bodies (LBs), found in the cytoplasm of neurons, often near the nucleus, and dystrophic neurites (Lewy neurites) found in axons and dendrites. LBs are intracytoplasmic aggregates of several proteins, including α-synuclein, ubiquitin, synphilin-1, 14-3-3 proteins, tubulin and other cytoskeletal proteins.

α-Synuclein can polymerize into ~ 10-nm fibrils in vitro and is the primary structural component of LBs [2, 3, 4]. In PD, these intracytoplasmic inclusions are distributed in the substantia nigra, locus coeruleus, medulla, olfactory bulb, and to a lesser extent in various cortical areas [5].

Familiar Forms of PD

Despite the overall rarity of the familiar forms of PD (<10% of the cases), the identification of single genes linked to the disease has yielded crucial insights into possible mechanisms of PD pathogenesis [for review see: 6, 7]. A role for α-synuclein in PD was fueled by the identification of a missense mutation in the α-synuclein gene as a cause of autosomal dominantly inherited PD [8]. Two mutations were identified, an Ala53Thr (A53T) mutation resulting from a G to A transition at position 209, and an Ala30Pro (A30P) mutation resulting from a G to C transition at position 88 [8, 9]. A genomic duplication or triplication of wild-type (wt) α-synuclein is the cause of the disease in some cases of familiar PD [10, 11]. Mutations in DJ-1 gene cause autosomal recessive PD. The large homozygous deletion of DJ-1 leads to protein loss, whereas a Lys166Pro point mutation destabilizes the protein. Recent structural studies indicate that DJ-1, which participates in the oxidative stress response by scavenging H₂O₂, may have similarities to the bacterial heat shock protein 31 (HSP31) homologs, suggesting that it may function as a chaperon to alleviate protein misfolding by interacting with early-unfolding intermediates [12, 13, 14]. Another familiar PD mutation affects ubiquitin carboxyl-terminal hydrolase-1 (UCHL1), a component of the cell ubiquitin-proteasome system (UPS) that degrades damaged proteins; UCHL1 has both hydrolase and ubiquitin ligase activities [15]. A more common causative mutation that involves the UPS affects an ubiquitin E3 ligase called parkin [16]. The Arg42Pro mutation in parkin causes autosomal recessive juvenile PD, possibly impairing parkin proteasomal interaction [17]. The Arg256Cys and Arg275Trp mutations cause altered protein localization and increased aggregation [18]. These mutations confer a loss-of-function of parkin’s E3 ligase activity; therefore, a great importance has been placed on the protein substrates of parkin. Parkin acts together with α-synuclein interacting protein synphilin-1, promoting the formation of

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LBs. Alternatively, parkin may interrelate with a minor O-linked glycosylated form of α-synuclein [19], and it has been hypothesized that parkin may act specifically on aberrant α-synuclein deposits (protofibrils/fibrils or oligomers). It was postulated that parkin rescues impaired proteasome function, thus preventing the toxic effects of mutant α-synuclein [20, 21]. Recently, a new familiar form of PD was identified, characterized by mutations in a putative mitochondrial protein kinase called PTEN-induced kinase 1 (PINK1) [22]. The missense mutation Gly309Asp is located in its ADP binding site, probably interfering with ADP binding and kinase activity, whereas the Trp437STOP mutation causes truncation of PINK1 in the helical kinase domain, probably destroying the enzyme. PINK1 contains a highly conserved kinase domain similar to serine/threonine kinases of the Ca2+-calmodulin family, which has been hypothesized to phosphorylate mitochondrial proteins in response to cellular stress, protecting against mitochondrial dysfunction [22].

PARKINSON’S DISEASE, A MISFOLDED PROTEIN DISORDER

α-Synuclein Protein

α-Synuclein mutations are autosomal dominant and represent a toxic gain-of-function mutation, resulting in abnormal protein accumulations [23, 24]. Even though mutations in α-synuclein are very rare causes of hereditary PD, its apparent role as the major structural feature of the LBs has placed α-synuclein at the center stage in PD pathophysiology [for review see: 25]. α-Synuclein is a 140-amino acid protein whose cellular function is still unknown. This protein is normally unfolded, revealing little three-dimensional structure. The α-synuclein non-amyloidogenic component (NAC) domain appears to be responsible for its aggregating properties and can form oligomers of β-sheeted sheets called protofibrils that can additionally form fibrils found in LBs and neuritis [26]. Moreover, it has been observed that protofibrils are toxic, and cells that contain LBs represent those that have managed to evade the toxic mechanisms involved in PD [27]. Several lines of evidence suggest that α-synuclein may play a role in vesicular function at synaptic level, since it is associated to vesicles and co-localizes with synaptophysin in presynaptic terminals [28]. Ostrerova and co-workers [29] proposed that α-synuclein could have a chaperone-like function since it has homology with 14-3-3 proteins. α-Synuclein appears to be associated with membrane compartments in cultured cells and brain tissue through interactions with acidic head groups of phospholipids [30]. Membrane-bound α-synuclein may play an important role in fibril formation [30, 31]. The molecular pathways of α-synuclein-mediated toxicity are unknown, but increased oxidative stress, mitochondrial injury and altered cellular transport have been proposed [7].

Fibrillization and aggregation of α-synuclein may be central in PD neuronal demise. The first evidence comes from the mutated α-synuclein familiar forms of PD where α-synuclein has an increased tendency to aggregate as compared to wt. There are several theories for in vivo α-synuclein aggregation in sporadic PD. Foremost mitochondrial dysfunction theory since complex I inhibition was shown to increase α-synuclein accumulation [32-36] (see Mitochondrial dysfunction section). Oxidative stress also seems to participate in this process [37], given that free radicals can induce α-synuclein aggregation (see Oxidative stress section). Proteasome dysfunction might also have a role in α-synuclein accumulation and aggregation [39, 40], since proteasome inhibitors induce α-synuclein aggregation (see Impairment in the proteasomal system section) (Fig. 1).

Moreover, Giasson and colleagues [38] reported that α-synuclein induces tau fibrillization in vivo and in vitro, and both proteins synergistically promote the polymerization of each other into fibrillar intracellular deposits. Both proteins can be phosphorylated, which facilitates their conversion into fibrils. The aggregation of tau and α-synuclein as fibrillar deposits can lead to their sequestration, thus putting them off from performing their functions [41].

Transgenic Models

To further elucidate the in vivo function of α-synuclein, several transgenic mice were developed. Knockout of the α-synuclein gene in mice resulted in a reduced level of dopamine in the striatum [42], reduction of the reserve pool of synaptic vesicles in the hippocampus and electrophysiological abnormalities, suggesting a role for α-synuclein in presynaptic regulation [43]. Dauer and co-workers [44] also generated α-synuclein null mice that were resistant to acute chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. Since 1-methyl-4-phenylpyridinium (MPP+), a mitochondrial complex I inhibitor, interacts with a variety of elements in the synaptic machinery, like the dopamine transporter, the vesicular monoamine transporter and vesicles, this outcome may reflect a participation of α-synuclein in vesicular function. An additional α-synuclein knockout mouse was generated by Schluter and collaborators [45] that had only a partial protection from MPTP-induced striatal dopamine loss.

Overexpression of wt human α-synuclein in mice resulted in loss of dopaminergic terminals, intranuclear and cytoplasmic ubiquitin inclusions in the substantia nigra, hippocampus, and cortex, and in motor deficit [46]. Expression of the A53T human α-synuclein in mice induced an early and dramatic decline of motor function [47]. Although the neuropathological assessment revealed the presence of α-synuclein, ubiquitin-positive inclusions and fibrillation of the microtubule-associated protein, tau, no modification in the nigrostriatal system was observed [47, 48].

In most of the transgenic models, the development of fibrillar α-synuclein inclusions seems to be associated with neurodegeneration. However, in one strain of transgenic mice, the parkinson’s phenotype appears to be associated with non fibrillar α-synuclein inclusions [46], raising the possibility that protofibrils may also have a role on the neurodegenerative pathway [49]. In contrast, Lee and colleagues [50] reported that A30P protofibrillogenic α-synuclein transgenic mice did not show neurodegeneration.

Synaptic Dysfunction

The role of α-synuclein in synaptic function has been described for its soluble form. Soluble α-synuclein is able to decrease the activity of tyrosine hydroxylase (TH), thus
modulating the amount of dopamine that is synthesized into the nerve terminal [51]. It can regulate the availability of synaptic vesicles and the reuptake of dopamine by the vesicular monoamine transporter 2 (VMAT2), therefore controlling catecholamine storage at nerve terminals [52, 53], and can reduce the maximal reuptake of dopamine from the extracellular synapse by dopamine transporter (DAT) [54, 55], modulating the time that dopamine remains in the synaptic cleft.

An alteration of α-synuclein synaptic function occurs upon its oligomerization into soluble protofibrils, and further to insoluble fibrils that may contribute to synaptic dysfunction.

Dopamine synthesis depends upon the conversion of tyrosine into L-dopa (levodihydroxyphenylalanine) by phosphorylated TH. The reduced availability of cytosolic α-synuclein, due to an increased aggregation state, might be responsible for the deleterious overproduction of cytosolic dopamine, generating an increase in highly reactive species such as quinones and superoxide free radicals [56].

α-Synuclein also plays a role in synaptic plasticity, since α-synuclein depleted cells exhibit a decreased expression in the levels of synapsin, an essential protein for synaptic vesicle recycling [43, 57]. Furthermore, α-synuclein inhibits the activity of phospholipase D2 (PLD2), which is involved in the building of vesicle from donor membranes and consequently, also controls vesicle formation and synaptic vesicles recycling [58]. It has been suggested that α-synuclein interferes with axonal transport of synaptic vesicles, by interaction with cytoskeleton proteins such as tubulin, tau, MAP2 and synphilin-1 [56]. In addition, the A53T mutant showed a decrease of dopamine release and vesicular dopamine storage through down regulation of VMAT2, probably due to a depletion of vesicular dopamine leading to its cytosolic accumulation [57]. Moreover, a decrease in VMAT2 labeling in patients with idiopathic PD has been described [59]. The toxic forms of α-synuclein can permeabilize vesicles, causing the leakage of small molecules, such as dopamine, to the cytosol [52].

In the terminals of dopamine-synthesizing neurons, α-synuclein modulates the functional activity of DAT. This transporter is responsible for the synaptic dopamine concentration and consequently, for the maintenance of dopaminergic neurotransmission. Wersinger and colleagues [54, 55] showed that α-synuclein markedly decreased DAT activity. Moreover, the same authors showed that α-synuclein interacted directly with DAT, modulating dopamine reuptake into the cytosol. Furthermore, A53T mutant was unable to modulate DAT function [55]. This shut down in DAT regulation could be responsible for the increase of dopamine in the cytosol, leading to dopamine-induced neurotoxicity.

**CELLULAR AND MOLECULAR PATHWAYS IN PARKINSON’S DISEASE**

**Deregulation of Neurotransmission**

The degeneration of dopaminergic neurons is combined with different degrees of alteration in GABAergic, glutamatergic, serotoninergic and norepinephrinergic systems...
considerable support for the free radical hypothesis, because electrons from neighboring molecules to complete their own electrons, are unstable reactive species that can extract free radicals. Free radicals, molecules with unpaired electrons, are reactive species that can extract electrons from neighboring molecules to complete their own orbital [67]. This leads to oxidation of cellular molecules, namely DNA, proteins, and membrane lipids.

The role of the nigrostriatal dopaminergic neuronal loss in the pathogenesis of PD has been questioned, since the hyperactivity of STN usually occurs before the appearance of parkinsonian signs, and is not correlated with inhibition of GPe activity. Indeed, the increased activity of STN, which is predominantly glutamatergic, might cause excitotoxicity and consequently, neuronal death [60, 62]. Glutamate is an excitatory neurotransmitter that can act on metabotropic and ionotropic receptors, such as NMDA, AMPA and KA receptors. Excitotoxicity can be the result of excessive activation of ionotropic receptors, mainly NMDA receptor, due to increased levels of glutamate in synaptic cleft. Overactivation of NMDA receptors can trigger a massive influx of extracellular Ca

Oxidative Stress

All aerobic organisms are continually exposed to oxidative stress. Oxidative and reductive reactions involve the transfer of electrons, which can lead to the generation of free radicals. Free radicals, molecules with unpaired electrons, are unstable reactive species that can extract electrons from neighboring molecules to complete their own orbital [67]. This leads to oxidation of cellular molecules, namely DNA, proteins, and membrane lipids.

The fact that age is a key risk factor in PD provides considerable support for the free radical hypothesis, because effects of the attacks by free radicals can accumulate over the years [68]. Moreover, dopamine metabolism and/or impairment of oxidative phosphorylation produce reactive oxygen species that contribute to an overall increase in oxidative damage.

There are several potential sources of oxidative damage in PD. Substantial data from post-mortem studies support an increased oxidative damage in PD. A consistent increase in lipid peroxidation and an increase in protein carbonyl groups have been reported in PD substantia nigra [69, 70]. A 138% increase in 8-hydroxy-2-deoxyguanosine concentration in DNA was observed in PD substantia nigra [71]. In addition, reduced glutathione, a very important antioxidant in the brain, is markedly decreased in the substantia nigra of PD patients [72]. A decrease in catalase and glutathione peroxidase activities in PD was reported, but superoxide dismutase activity in substantia nigra of PD patients was shown to be increased [73, 74]. Also relevant is the fact that the iron levels, which in a non-pathological situation are significantly higher in substantia nigra than in other brain regions, further increase in substantia nigra of PD patients [74].

Nigral dopaminergic neurons are particularly exposed to oxidative stress, since dopamine can easily auto-oxidize into toxic dopamine-quinone species, superoxide radicals and hydrogen peroxide [75]. Moreover, dopamine can be metabolized via enzymatic deamination by monoamine oxidase (MAO), with the production of the non-toxic 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide [76]. Superoxide radical can be converted to hydrogen peroxide by superoxide dismutase or into the more toxic peroxynitrite radical in the presence of nitric oxide. Hydrogen peroxide can be rapidly converted to a more reactive radical, hydroxyl radical, in a reaction catalyzed by iron, which is highly abundant in substantia nigra pars compact [77]. The loss of protein sulphhydryl groups induced by dopamine toxicity is associated with the loss of monoaminergic striatal terminals [78]. Oxidized dopamine has been shown to stabilize the formation of α-synuclein protofibrils that are claimed to be the more toxic form of α-synuclein [79], inhibiting its conversion to insoluble fibrils [80].

A dual relationship exists between α-synuclein and the production of free radicals. Not only can the oxidative process can transform non-aggregated α-synuclein into its aggregated form, but also by itself may be able to generate hydrogen peroxide in vitro [81]. Therefore, it could be speculated that once an oxidation process starts, an enhancement of α-synuclein aggregation occurs leading to an increased neurotoxicity, with release of cellular iron, which catalyzes further oxidations, initiating a positive feedback of oxidative neurodegeneration. Moreover, high expression levels of the wt and mutant forms of α-synuclein cause an increase in reactive oxygen species (ROS) production [82]. Also important is the fact that α-synuclein toxicity in cultured neurons is blocked by antioxidants [83].

Further evidence that oxidative stress participates in the loss of nigral neurons in PD was obtained from studies using the parkinsonism-inducing drugs MPP+ and rotenone. MPP+ is taken up into dopaminergic neurons by DAT, and in addition to inhibiting complex I of the mitochondrial
mitochondrial membrane potential. However, upon rotenone treatment, both wt and mutated α-synuclein cells showed a decrease in mitochondrial membrane potential, indicating a correlation between mitochondrial and α-synuclein. In addition, it has been shown that α-synuclein expression in mice substantia nigra was insufficient to produce mitochondrial pathology, which could be observed with MPTP supplementation [95].

**Impairment in the Proteasomal System**

An involvement of the proteasomal system in PD has been described, partially because mutations in UCHL1 and parkin showed a contribution to neurodegeneration in PD. Moreover, a decrease in the proteasome peptidase activity in the substantia nigra of sporadic PD patients has been described [96, 97]. McNaught and co-workers [97] also showed a decrease in the expression of proteasomal α-subunits in substantia nigra of PD patients. More recently, Grüblatt and collaborators [98] showed modifications of the gene expression profile in ubiquitin-proteasome system in substantia nigra pars compacta of sporadic PD patients. These data are in accordance with McNaught and colleagues [99] reports showing a decreased expression of the 20S proteasome α-subunits, decreased protein expression levels of some 19S subunits and functional deficits in the 26/20S proteasome activity in substantia nigra pars compacta of sporadic PD patients. Other studies correlate α-synuclein with proteasome function, since α-synuclein can interact with a subunit of the proteasome regulatory complexes [100], and proteasome subunits co-localize in LBs [101]. It has been demonstrated that proteasome pathway may mediate the degradation of α-synuclein. This degradation, proposed by several authors [102, 103, 104], seems to be mediated by the 20S proteasome, indicating that impairment in the proteasome activity could promote α-synuclein accumulation and further aggregation. These conclusions were supported by several in vitro studies demonstrating that proteasome inhibitors promote α-synuclein aggregation [103, 105, 106]. On the other hand, it has been shown by several groups that α-synuclein overexpression produces a decrease of proteasome peptidase activity [107, 108]. A decrease in proteasome activity in PC12 cells expressing mutant α-synuclein was also described [109]. Taken together, these results suggest that α-synuclein overexpression may lead to proteasome inhibition, creating a positive feed-back loop, that will increase α-synuclein accumulation and aggregation. In agreement with in vitro studies is the observation that a duplication or triplication of the α-synuclein gene leads to PD [10, 11]. Nevertheless, this issue is still controversial since it has been described that α-synuclein expression either in vivo (transgenic mice for human α-synuclein A30P) and in vitro (PC12 cells overexpressing wt A30P and A53T α-synuclein), did not affect proteasome function, measured by proteasome peptidase activities or proteasome subunits expression [110]. Moreover, the well-known complex I inhibitors, MPP+ and rotenone, induce a proteasome activation in rat mesencephalon primary culture [106]. It has been demonstrated that proteasome inhibitors render cells more vulnerable to death [109, 111, 112]. On the other hand, Sawada and colleagues [106] showed that proteasome inhibition blocked MPP+ or rotenone induced cell death.

**Mitochondrial Dysfunction**

Mitochondria are the intracellular organelles responsible for the supply of ATP; they are semiautonomous, they contain their own DNA and protein synthesizing machinery, although most of the proteins that reside in the mitochondria are nuclear gene products [84]. Defects in the electron transport chain within the mitochondria are major factors contributing to the production of free radicals. It has been described that mitochondrial function is altered in the course of aging and also in PD, particularly an alteration in the mitochondrial respiratory chain complex I [85]. Many studies have shown that mitochondrial dysfunction is implicated in the pathogenesis of PD [8, 86, 87, 88]. Two consistently identified biochemical abnormalities in PD substantia nigra are mitochondrial complex I deficiency and increase in free radical production [25, 89]. The complex I defect in idiopathic PD is of particular interest, since inhibitors of this complex cause nigral cell death in humans and in animals.

Co-enzyme Q10 (CoQ10), which improves electron respiratory chain function and scavenges free radicals, has been described to exist at a lower concentration in patients with PD [90], probably due to the complex I defect observed in these patients [91].

Parkinsonism was induced in humans by MPP+, a metabolite of MPTP [92], and more recently, the pesticide rotenone was shown to induce neurodegenerative changes similar to PD in rats [36], both molecules being complex I inhibitors. Further support of mitochondrial involvement in the pathogenesis of PD was obtained using a cybrid technique, that uses cell lines without a functional mitochondria fused with mitochondria from platelets (or other tissues) obtained from PD patients. Cassarino and collaborators [93] observed a decrease in complex I activity and an increased production of free radicals in PD cybrids, suggesting that the decrease in complex I activity present either in PD brains or platelets, can be responsible for the damaging amounts of free radicals observed. Complex I deficiency and free radical damage are inter-related, since a defect in complex I causes an increase in the release of superoxide ions from the respiratory chain and, in turn, free radicals increase leads to an impaired activity of respiratory chain proteins. Much of the cellular damage caused by rotenone in rodents seems to be mediated not by ATP depletion but by generation of free radicals [36].

Moreover, the relationship between mitochondrial function and α-synuclein expression has been studied to address the possible pathological mechanism of PD. Orth and colleagues [94] showed that mutant or wt α-synuclein did not alter mitochondrial respiratory chain activity or mitochondrial membrane potential. However, upon rotenone...
Chaperones

Molecular chaperones are involved in several important cellular processes. They bind unfolded proteins and can either mediate correct folding and compartmentalization or proteasomal degradation. This includes normal proteins that are intended for deactivation or abnormal proteins that are mislocalized or are defective, due to misfolding or chemical modification, and whose accumulation could be toxic [113].

It is generally accepted that PD is a protein conformational disease, being the hallmark event a change in its structure. Recently, it has been described that chaperones may play a role in this conformational change. Furthermore, immunostaining studies showed the presence of chaperons in LBs of human postmortem tissue, also suggesting that chaperones may play a role in Parkinson’s disease progression [114]. Most studies in PD focus on the role of chaperones in α-synuclein fibril formation, and it has been proposed that the expression of small heat shock proteins (sHsp) reflect a defensive response to diminish α-synuclein fibril formation and subsequent toxicity. It was demonstrated that the molecular chaperone Hsp70 could reduce the amount of misfolded, aggregated α-synuclein species in vivo, therefore protecting cells from α-synuclein-dependent toxicity [115]. Additionally, it has been demonstrated in vitro that over expression of the heat shock protein HSP27 has a protective anti-apoptotic outcome against the toxic role of wt and particularly of mutant α-synuclein. On the other hand, HSP70 can protect from the toxic effect of the wt protein, but has no action against the mutant proteins, while HSP56 has no protective effect in this system [116].

NEUROINFLAMMATION

The hallmark of brain inflammation, also designated as neuroinflammation, is the activation of glial cells, mainly microglia. An intense glial (astrocytes and microglia) activation was observed in substantia nigra of PD patients [117] and in PD animal models [118]. A large number of recent studies postulate that microglia and astrocytes play a key role in progressive degeneration of DA neurons in PD. Microglia, the immune effector cells of the brain, are sensitive to minor disturbances in brain homeostasis, and become activated during injurious or neuropathological conditions. Although these cells can have a neuroprotective function by the secretion of neurotrophic factors and elimination of cellular debris and/or pathogens, activated microglia can also trigger neuronal damage via release of pro-inflammatory and neurotoxic factors. These factors include the pro-inflammatory cytokines, interleukin 1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), T-cell activation-associated cytokine (IL-2), interferon-γ (IFNγ), ROS and reactive nitrogen species, (RNS), proteases and acute phase proteins. Astrocytes can be activated by inflammatory or neurotoxic factors released by reactive microglia and/or injured neurons. Reactive astrocytes secrete, primarily, neurotrophic factors, but they can also produce inflammatory and neurotoxic factors similar to those secreted by activated microglia. Therefore, both glial cells, depending on the degree of activation, can influence the fate of the injured neurons. In PD brain, the dying DA neurons and the consequent biochemical environment changes can be the trigger of microglia and astrocyte activation [119, 120]. Microglia can also be activated by products of the classical component cascade, such as C-reactive protein, that is upregulated in PD brain [121] Pro-inflammatory cytokines once released from reactive glial cells, can bind to their receptors on DA neurons to activate transduction pathways that trigger apoptosis. In addition, the substances derived from activated glial cells can trigger the expression of inducible nitric oxide synthase (iNOS) or cyclooxygenase-2 (COX-2) within these cells, thus leading to reactive nitrogen and oxygen species production. These reactive species can cross the cell membrane and cause oxidative damage, especially in DA neurons that are extremely vulnerable to oxidative stress. An intense gliosis was observed around DA neurons in PD brain, despite a long (3-16 years) neuronal survival, and it was postulated that after a primary neuronal insult of environmental or genetic origin, glial activation might perpetuate the degeneration of DA neurons [122].

MECHANISMS OF CELL DEATH

Depending upon the stimuli, cells might die by necrosis, apoptosis or autophagy. It is still not clear what type of cell death occurs in PD brains, although the slow and progressive degeneration of dopamine neurons is more compatible with apoptosis. The cell death of dopaminergic neurons in substantia nigra pars compacta causes a remarkable loss of dopamine in caudate/putamen nuclei.

Necrotic cell death exhibits cytoplasmic and nuclear swelling, membrane breakdown, leading to the leakage of cytosolic contents and inflammation. Necrosis may occur in PD when neurons are exposed to high glutamate concentrations, involving an excitotoxic pathway [123]. Although there is no available data in literature describing necrotic cells in substantia nigra of PD patients, the hypothesis of their existence cannot be disregarded, since their rapid elimination may constitute the reason why they are not detected.

Autophagic cell death is characterized by the vacuolation of the cytosol within a double-membrane structure, the autophagosome, which fuses with the lysosome for subsequent digestion [124]. As a consequence, the destruction of the cytoplasm occurs, maintaining the integrity of the nucleus (in contrast to apoptosis) and of the plasma membrane (in contrast to necrosis). This kind of cell death has been observed in nigral neurons of PD patients [125]. Gómez-Santos and co-workers [126] showed that dopamine induced autophagic cell death in a human neuroblastoma cell line. An autophagic cell death in PC12 cells expressing the A53T α-synuclein mutant has also been described [127]. It is relevant to elucidate that apoptotic signals may also activate autophagy as an alternative mechanism of cell death [128]. Recently, it was demonstrated by Rideout and collaborators [129] that proteasomal inhibition in primary neurons leads to autophagy, possibly in response to the apoptotic pathway.

Apoptotic cell death depends on different interacting signaling pathways, and diverse insults may activate alternative pathways leading to nuclear chromatin condensation and DNA fragmentation. Apoptotic cells show
membrane blebbing, DNA fragmentation and do not induce immune response. Apoptosis occurs during normal cell development but can also be responsible for neuronal death in some diseases, and there is increasing evidence that it is also involved in PD. [130]. Apoptotic features have been shown in PD patients, like fragmented nuclei and caspase activation [131, 132, 133]. Moreover, it has been described that pro-apoptotic genes (c-Jun, P53, GAPDH, Bax) are up regulated in substantia nigra of PD patients [134], and five fold higher caspase 3 positive neurons were found in patients as compared to controls [132]. This subject is still controversial, since in the end-stage of the disease, there is little evidence of apoptosis [135, 136]. In addition, it was demonstrated that 6-hydroxydopamine (6-OHDA), a dopamine quinone derivative, present in post-mortem PD brains [137], upregulates c-Jun and activates caspase 3 in vitro [138].

In vitro studies have shown that chronic administration of rotenone in a neuroblastoma cell line increases basal and H2O2 induced caspase dependent cell death [34]. It has been demonstrated that MPP+ induced mitochondrial cytochrome c release in a human neuroblastoma cell line [139], and apoptotic cell death both in vitro [140] and in vivo [141, 142, 143].

Recently, Yamada and co-workers [144] observed that overexpression of α-synuclein in rat substantia nigra induced a 50% loss of dopaminergic neurons and caspase 9 activation. Moreover, the study performed by Watabe and Nakaki [145] showed that rotenone induces neuronal death in SH-SY5Y cells via Bad dephosphorylation and caspase 9 activation.

THERAPEUTIC STRATEGIES

Drugs Involved in Neurotransmission Improvement

L-dopa: Replacement of striatal dopamine with the precursor of dopamine, L-dopa, is the most effective pharmacological treatment of PD. Since dopamine itself could not access the brain directly, its natural precursor L-dopa was used in clinical trials. By 1967, Cotzias and co-workers [146] were able to announce that large oral doses of L-dopa resulted in a therapeutic success in the treatment of the disease. However, it was discovered that although its initial results were dramatically effective, a growing tolerance developed, resulting in a need to increase dosages over time. Eventually, side effects resulting from high doses of the drug, such as dyskinesias, gastrointestinal symptoms, insomnia, hallucinations and psychosis, outweigh any improvement observed from the correction of dopamine deficiency [147]. It was suggested that the damaging side effects of L-dopa’s use stem not directly from the drug but from its oxidation products, which include dopachrome and other indoles which are hallucinogenic, toxic to neurons and have been shown to hasten death in PD patients [75, 148].

Several new dopaminergic drugs are in advanced clinical development and will be released soon to the market: sumaniprole, rotigotine, L-dopa methylster and ethylster, and the triple combination of L-dopa/carbidopa/entacapone, Piribedil [149].

Drugs Acting at the Dopamine Transporter Level: These drugs increase the synaptic levels of dopamine by blocking the action of the synaptic dopamine transporter. Brasofensine is one of these drugs, producing a potentiation of the effects of L-dopa in MPTP-treated monkeys without inducing dyskinesia [150]. In 8 patients receiving the drug, an initial improvement over a 4-week period was observed, but by the end of the study, this improvement had been lost, and no significant differences were shown against placebo [150].

Selective Dopamine Receptor Agonists: D1 receptors have been repeatedly involved in the origin of L-dopa-induced dyskinesias, and selective D1 receptors agonists have been shown to be highly effective in MPTP-treated monkeys, producing a natural motor response without dyskinesia [151, 152]. Dihydrexidine, a high-affinity full D1 agonist, produced a dramatic reduction of parkinsonian signs in severely afflicted MPTP-treated monkeys [153]. Furthermore, Blanchet and co-workers [154] performed a clinical trial where 4 patients received dihydrexidine intravenously. Although three patients were withdrawn from the study due to severe postural hypotension, the remaining patient showed a 70% improvement in Unified Parkinson’s Disease Rating Scale (UPDRS) score.

ABT-431, another D1 agonist, showed positive effects against parkinsonism and dyskinesia in MPTP-treated monkeys [155]. However, when tested in humans with PD and motor complications, ABT-431 had a similar action to L-dopa, but without significant modification of dyskinesia [156, 157].

Partial Dopamine Receptor Agonists: This class of compounds exerts agonist activity at denervated supersensitive sites and competitively inhibits complete agonists at fully innervated normosensitive receptors. Thus, their therapeutic window appears to be higher than full agonists mainly in patients with L-dopa-induced dyskinesia or psychiatric disturbances. Terguride, a partial D2 receptor agonist, exhibited both antiparkinsonian and antidyskinesia effects in MPTP-treated monkeys [158], but when administered to patients with motor complications, the results were inconsistent. In some cases, the drug reduced dyskinesia without worsening parkinsonism but, in others, it worsened both dyskinesia and PD symptoms [159].

Non-dopaminergic drugs: Other pharmacological strategies include adenosine A2a receptors antagonists, α2 noradrenergic receptor antagonists, GABAergic drugs, drugs acting on serotoninergic transmission, glutamate antagonists, cannabinoids agonists and antagonists and opioid receptor agonists and antagonists [for review see: 149]. Table I shows the effects of some of these agents in animal and human studies.

The efficacy of the drugs above mentioned is lower than that of L-dopa, but their initial or combined use with L-dopa might help to avoid or delay the occurrence of drug-induced dyskinesias and neuropsychiatric adverse effects, which often complicate the medical treatment of PD [191]. These complications have prompted many additional strategies to lessen drugs effects, for example, with continuous drug infusions by transdermal, intravenous or intraduodenal drug-delivery systems [192].
Antioxidant Therapies

Co-enzyme Q is an essential co-factor of the electron transport chain (accepts electrons from complexes I and II) and possesses antioxidant properties. Beal and colleagues [193] reported that the administration of CoQ10 to 24-month-old mice treated with MPTP induced a significant protection against neuronal dopamine depletion and loss of tyrosine hydroxylase. Muller and collaborators [194] performed a monocenter, parallel, placebo controlled, double-blind trial aimed to determine the symptomatic response of daily oral application of 360 mg CoQ10 on scored PD symptoms in 28 treated and stable PD patients. They observed a significant mild symptomatic benefit and a better improvement of performance compared with placebo. Recently, the safety and tolerability of high dosages of CoQ10 were studied in 17 patients with PD in an open label study [195]. The subjects received an escalating dosage of CoQ10 (1200, 1800, 2400, 3000 mg/day) with a stable dosage of vitamin E (1200 IU/day). 13 patients achieved the maximal dosage without adverse effects related with CoQ10. Furthermore, the plasma level reached a plateau at the 2400 mg/day dosage and did not increase further at the 3000 mg/day, dosage suggesting that in future studies of CoQ10 in PD, a dosage of 2400 mg/day (with 1200 IU/day vitamin E) is an appropriate highest dosage to be studied.

Recently, Zhang and co-workers [196] reported the results of a prospective study, which showed that high dietary intake of food rich in vitamin E is associated with a reduced risk of developing PD. However, no protective effect was observed with supplemental vitamin E or C, or with dietary vitamin C or carotenoids. The Parkinson Study Group [183] tested vitamin E at a daily dose of 2000 IU in a prospective, placebo-controlled, double blind trial of 800 previously untreated patients. The investigators simultaneously tested the effect of deprenyl (MAOB inhibitor) and vitamin E by the use of a 2x2 factorial design. The results obtained showed that vitamin E failed to delay the rate of disease progression compared with placebo, whether administered alone or in combination with deprenyl. These results are supported by a recent study showing that vitamin E is ineffective, because it did not enhance the activity of nigrostriatal dopaminergic neurons [197].

Ginkgo biloba has been reported to protect dopamine neurons from MPTP-induced neurotoxicity [198]. Recently, Kim and collaborators [199] demonstrated that EGB 761 (extract of Ginkgo biloba) reduces the behavioral deficit caused by 6-hydroxydopamine-induced neurotoxicity in the nigrostriatal dopaminergic system of the rat brain. Furthermore, it has been demonstrated in a rat model of PD that the combined use of EGB with L-dopa may be a workable method to treat PD and may be better than using L-dopa alone [200].

Estrogens

Accumulating scientific evidence of estrogen beneficial effects on dopaminergic neurotransmission, as well as increased interest in estrogens role in cognition and dementia, led to renewed efforts to study its effects in PD. Two recent investigations have demonstrated that estrogen administration is beneficial for the symptoms of PD [201, 202]. The double-blind trial performed by Tsang and co-workers [202] showed a significant improvement in motor function in postmenopausal women taking estrogen as compared with placebo treatment. In the second study, Saunders-Pullman and colleagues [201] conducted a retrospective chart review of 138 women who had PD for less than 5 years and who had not taken L-dopa. They found a positive association between estrogen use and reduced severity of symptoms. However, results from other studies have been less clear about the benefits of estrogen in ameliorating PD symptoms. In two cases reported in Lancet [203], oral conjugated estrogens abolished dyskinesia while aggravating PD symptoms, suggesting an antidopaminergic effect. However, the study performed by Blanchet and collaborators [204] showed that 17β-estradiol appears to display a slight pro-dopaminergic effect without consistently altering dyskinesias.

Cultured mesencephalic neurons incubated in medium containing estradiol were resistant to bleomycin sulfate-induced apoptosis [205]. The effect induced by estradiol was blocked by the specific estrogen receptor antagonist ICI 182,780, which suggests that the neuroprotective effect was mediated by classical estrogen receptors [205]. Preincubation with estradiol similarly provided significant neuroprotection against glutamate-induced neurotoxicity [206]. It has been shown that the direct infusion of 6-hydroxydopamine (OHDA) into the striatum of ovariecotomized rats significantly reduced the dopamine concentrations within the lesioned side [207]. However, animals that had been implanted with a time-release estrogen pellet, underwent

Table 1. Effect of Some Non-Dopaminergic Agents in Animal Models and PD Patients

<table>
<thead>
<tr>
<th>Non-dopaminergic agents</th>
<th>Positive effects</th>
<th>Negative or no effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A2a receptor antagonists</td>
<td>[160, 161, 162, 163]</td>
<td>[164]</td>
</tr>
<tr>
<td>α2 noradrenergic receptor antagonists</td>
<td>[165, 166, 167]</td>
<td>[167]</td>
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<tr>
<td>GABAergic drugs</td>
<td>[168, 169, 170, 171, 172]</td>
<td>[173, 174, 175, 176]</td>
</tr>
<tr>
<td>Drugs acting on serotonergic transmission</td>
<td>[177]</td>
<td>[164, 178]</td>
</tr>
<tr>
<td>Glutamate antagonists</td>
<td>[179, 180, 181, 182]</td>
<td>[183, 184]</td>
</tr>
<tr>
<td>Cannabinoids agonists and antagonists</td>
<td>[185, 186]</td>
<td></td>
</tr>
<tr>
<td>Opioid receptor agonists and antagonists</td>
<td>[187, 188]</td>
<td>[189, 190]</td>
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significantly less dopamine depletion after injection of OHDA [207]. Recently, Shughrue [208] showed that mice pretreated with estradiol present less MPTP-induced tyrosine hydroxylase loss when compared with untreated mice. Quesada and Micevych [209] reported that pretreatment of ovariectomized female mice with estrogen or insulin growth factor 1 (IGF-1) significantly prevented 6-OHDA-induced loss of substantia nigra compacta neurons (20% loss) and tyrosine hydroxylase immunoreactivity in dopamine fibers in the striatum (<20% loss) and prevented the loss of asymmetric forelimb use. Blockage of IGF-1 receptors by intracerebroventricular JB-1, an IGF-1 receptor antagonist, attenuated both estrogen and IGF-1 neuroprotection of nigrostriatal dopaminergic neurons and motor behavior. These findings suggest that IGF-1 and estrogen acting through the IGF-1 system may be critical for neuroprotective effects of estrogen on nigrostriatal dopaminergic neurons in this model of Parkinson’s disease.

Non-steroidal Anti-Inflammatory Drugs (NSAID)

The possible anti-inflammatory strategy against neurodegeneration in PD is supported by experimental studies using some NSAID on in vitro and in vivo models of PD. It has been shown that the pretreatment with sodium salicylate, aspirin and meloxicam, a preferential COX-2 inhibitor, significantly and almost completely protected MPTP-induced striatal dopamine depletion, locomotor activity and loss of nigral dopaminergic neurons [210-213], although other non-selective NSAID (paracetamol, diclofenac, ibuprofen, indomethacin) and dexamethasone showed no protective effects [210]. Breidert and colleagues [214] showed that oral administration of pioglitazone, a PPARγ agonist, attenuated the MPTP-induced decrease in striatal dopamine levels and dopaminergic cell loss in the substantia nigra [214]. Recently, Chen and collaborators [215] observed that individuals who reported regular use of non-aspirin NSAID at the beginning of the study had a lower risk of PD than non-regular users during the follow-up. Compared with non-users, a non-significant lower risk of PD was also observed among individuals taking 2 or more tablets of aspirin per day. These results suggest that the use of NSAID may delay or prevent the onset of PD.

Surgical Treatment

The efficiency of L-dopa declines over time in a majority of patients for whom motor fluctuations and L-dopa-induced dyskiniesias become frequent [191]. This fact, together with refined stereotaxic procedures, sophisticated brain-imaging techniques and advanced computer programs, has led to a resurgence of surgical methods for the treatment of PD. Ablative techniques, deep brain stimulation, and neural transplantation are now used to correct the striatal loss of dopamine and related functional disturbances in the basal ganglia circuitry [192, 216, 217].

Neural Transplantation: The efficacy of cell replacement for the treatment of PD is based on two hypothesis: 1) the predominant symptoms develop because of the dysfunction or loss of the dopaminergic neurons in the nigrostriatal pathway; 2) dopaminergic neurons grafted into the dopamine-deficient striatum can replace the neurons lost as a result of the disease process and can reverse, at least in part, the major symptoms of the disease [218, 219]. Lindvall et al. [219] reported that mesencephalic dopamine neurons, obtained from human fetuses of 8 to 9 weeks gestational age, could survive in the human brain and produced marked and sustained symptomatic relief in a patient severely affected with idiopathic PD. Furthermore, experiments in 6-OHDA-lesioned rats with L-dopa-induced dyskinesia have shown that grafting dopaminergic fetal cells into the striatum produced an improvement in motor behavior, including dyskinesia, which was accompanied by a reversal of the molecular mechanisms theoretically responsible for dyskinesia [220]. Paradoxically, two recently published double-blind studies using this technique have not been able to demonstrate significant efficacy and were both complicated by the occurrence of off-medication dyskinesias [217, 221, 222]. The use of this therapeutic strategy is furthermore hampered by ethical and practical considerations, owing to the need for large amounts of immature dopaminergic nervous tissue (three to four fetuses for each side of the brain, obtained at a very narrow gestational age) due to an estimated 10% survival rate of the transplanted tissue and the lack of consensus regarding the implantation technique.

The rationale for the use of drugs that will improve the viability of donor cells and/or transplanted cells is that such pharmacological treatment will result in a better functional outcome. Neurotrophic factors are potential therapeutic agents that could enhance viability and functional effect of neural transplantation therapy. Hoffer and colleagues [223] initially demonstrated that a single intraventricular injection of glial-cell-derived neurotrophic factor GDNF to a normal rat induces a long-term increase in striatal dopamine. Most critically, GDNF has been able to prevent the structural and/or functional consequences that are engendered in virtually every rodent model of PD including those with lesions induced by OHDA, methamphetamine, or axotomy as well as the degenerative changes associated with aging (for review see: 224). Based on these studies, experiments were performed in normal and parkinsonian monkeys, and similar benefits of GDNF treatment were realized [225]. This preclinical demonstration of the efficacy of GDNF in animal models of PD has prompted small clinical trials of such therapy in patients. In a PD patient who received monthly intraventricular injections of GDNF, parkinsonian symptoms continued to worsen [224]. These observations support the contention that GDNF might only be efficacious when initiated in early stage PD. Moreover, in this GDNF-treated patient, there was no evidence of restoration of nigrostriatal neurons and no indication of intraparenchymal diffusion of the GDNF to relevant brain regions. These findings suggest that the route of delivery (intraventricular) of GDNF is not an effective approach. Recently, Gill and co-workers [226] reported on the direct lentiviral delivery of GDNF into the putamen, the brain area with the most severe dopamine depletion, in five patients with PD. The effects on parkinsonism, openly assessed, were highly beneficial. After 1 year, there was a 39% improvement in the off-treatment motor score and a 61% improvement in the activity of daily living score of the unified PD rating scale. Drug-induced dyskinesias were reduced by 64%. Fluorine-18-labeled dopamine uptake was increased in the putamen, which
suggests a direct effect of GDNF on dopamine function. Similarly, it has been shown that transplantation of human pigmented epithelial retinal cells [227] have led to remarkable improvements in a few PD patients. However, these studies need to be confirmed in controlled trials.

**Ablative Technique:** Disruption of the proposed hyperactive STN or the increased inhibitory output from GPI by localized stereotaxic lesions, aided by advanced brain imaging, microelectrode recordings and pre-lesional stimulation of the target (macrostimulation), has been shown to be an effective and reasonably safe procedure [216]. These interventions alleviate contralateral L-dopa-induced dyskinesias and improve parkinsonian rigidity, tremor and to a lesser extent akinesia, however, misplaced lesions might cause irreversible neurologic, cognitive and neuropsychiatric adverse effects [216].

**Deep brain stimulation:** The hyperactive pathways from GPI and STN might also be modulated by implanted electrodes, which block the neural activity in their vicinity with a high-frequency electrical current [192, 216]. This procedure is called deep brain stimulation (DBS) and has proven very efficient in the treatment of PD complicated by motor fluctuations and L-dopa-induced dyskinesia [228]. Recently, the effects of DBS of the STN were studied in 52 consecutive patients (13 over age 70, 15 under age 60, 24 age 60 to 70). All groups showed improvement of motor fluctuations and dyskinesia. Patients age 70 had worsening of the UPDRS motor scores on medication, despite less medication reduction. Their activities of daily living and axial succors worsened, particularly in those with preoperative gait difficulties. Rodriguez-Oroz and collaborators [229] evaluated the long term (4 years) efficacy of DBS of the STN in advanced PD. DBS provided a significant and persistent anti-parkinsonian effect in advanced PD 4 years after surgery. Another study indicates that bilateral STN activates the projection of axons from the STN, improving both ascending and descending pathways from the basal ganglia and increasing the metabolism of higher-order motor control in the frontal cortex.

DBS hereby represents a more flexible method for the modulation of basal ganglia circuitry than ablative surgery, and can be used bilaterally without the same occurrence of neuropsychiatric and cognitive adverse effects [192, 216], although a few cases of severe mood changes and slight deficits in language abilities have been noted postoperatively [230]. These adverse effects are probably related to the influence of the electrode current on the limbic, associative, and cognitive corticobasal ganglia-thalamo-cortical loops. The most common adverse effects are however, related either to the surgical implantation, which might cause hemorrhage infections or seizures, or to the influence of the electrical current on neighboring corticobular projections, which might result in dyskinesia, diplopia, and paresthesias [228].

**New Promising Strategies**

It has been reported that F3 human multipotent neural stem cells (NSC) genetically engineered to produce L-DOPA by double transfection with cDNA for tyrosine hydroxylase and guanosine triphosphate cytohydrolase-1, and transplantation of these cells in the brain of Parkinson disease model rats led to L-DOPA production and functional recovery [231]. Proactively transplanted F3 human NSC in rat striatum, supported the survival of host striatal neurons against neuronal injury caused by 3-nitropro-pionic acid in an rat model of Huntington’s disease. Furthermore, intravenously introduced through the tail vein, F3 human NSC were found to migrate into ischemic lesion sites, differentiate into neurons and glial cells, and improve functional deficits in rat stroke models [231]. These results indicate that human NSC should be an ideal vehicle for cell replacement and gene transfer therapy for patients with neurological diseases. In addition to immortalized human NSC, immortalized human bone marrow mesenchymal stem cell lines have been generated from human embryonic bone marrow issues with retroviral vectors encoding v-Myc or telomerase gene. These immortalized cell lines of human bone marrow mesenchymal stem cells differentiated into neurons/glial cells, bone, cartilage and adipose tissue, when they were grown in selective inducing media [231]. There is further need for investigation into the neurogenic potential of the human bone marrow stem cell lines and their utility in animal models of neurological diseases. Zhao's Group [232] provide evidence for the generation of dopaminergic projection neurons of the type that are lost in Parkinson's disease from stem cells in the adult rodent brain and show that the rate of neurogenesis is increased after a lesion. The number of new neurons generated under physiological conditions in substantia nigra pars compacta was found to be several orders of magnitude smaller than in the granular cell layer of the dentate gyrus of the hippocampus. These data indicate that neurogenesis in the adult brain is more widespread than previously thought and may have implications for our understanding of the pathogenesis and treatment of PD. Recently, Hoglinger and collaborators [233] provided ultrastructural evidence showing that highly proliferative precursors in the adult subependymal zone express dopamine receptors and receive dopaminergic afferents. The authors observed that experimental depletion of dopamine in rodents decreases precursor cell proliferation in both the subependymal zone and the subgranular zone being the proliferation restored completely by a selective agonist of D2-like (D2L) receptors. In the same study, the authors observed that the activation of D2L receptors in neurosphere cultures, obtained from neural precursors from the adult subependymal zone, directly increases the proliferation of these precursors. Consistently, Hoglinger and co-workers [233] observed that the numbers of proliferating cells in the subependymal zone and neural precursor cells in the subgranular zone and olfactory bulb are reduced in postmortem brains of individuals with PD. These observations suggest that the generation of neural precursor cells is impaired in PD as a consequence of dopaminergic denervation. In the same line, Van Kampen and collaborators [234] demonstrated a two-fold induction of cell proliferation (BrdU incorporation) in the subventricular zone and rostral migratory stream of the adult Sprague-Dawley rat brain following intrasubventricular administration of the dopamine D(3) receptor agonist, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) for 2 weeks. The number of BrdU-positive cells was elevated ten-fold from very low baseline levels in the neighboring neostriatum, another region known to express D(3) receptors. These striatal BrdU-positive cells appeared within 3 days following intracerebral
infusion of 7-OH-DPAT and were distributed homogeneously throughout the striatum following systemic administration. This suggests that these cells were originated from resident progenitor cells rather than the subventricular zone. Furthermore, dopamine D(3) receptor activation may serve as a proneuronal differentiation signal, since 60-70% of the new cells had neuronal markers following 7-OH-DPAT infusion. These results suggest that the dopamine D(3) receptor may be a good drug target for cell replacement strategies, particularly because of the fact that its expression is almost exclusively limited to the nervous system.

Furthermore, it has been shown that the subthalamic injection of viral vectors expressing glutamic acid decarboxylase (converts glutamate to GABA) can induce the hyperactive excitatory glutamatergic cells in the STN to express GABAergic inhibitory responses. It has been shown that this alteration reversed a parkinsonian behavioral phenotype in rats [235].

These approaches remain experimental therapies that would require rigorous research efforts and clinical trials to validate their potential for treatment of PD (Fig. 2).

Fig. (2). Schematic drawing illustrating the current pharmacological and surgical therapeutical strategies employed in PD. Pharmacological treatment encloses several drugs acting at different levels: 1) dopaminergic drugs (such as precursors of dopamine, dopamine selective/partial receptor agonists and inhibitors of MAOB and COMT); 2) antioxidants and estrogens primarily involved in fighting oxidative stress and NSAID aimed to avoid inflammatory processes. Surgical treatment has emerged as an efficacious experimental treatment for PD. The logistical and ethical issues that impede large-scale clinical trials with human fetal cells as donor cell grafts led the scientists to explore other strategies such as genetically engineered cells, stem cells and/or immortalized cell lines that express specific dopaminergic or neurotrophic factors, direct delivery (viral) of neurotrophic factors, ablative surgery and DBS. For more detail see text.
ABBREVIATIONS

6-OHDA = 6-hydroxydopamine  
Ach = Acetylcholine  
CoQ10 = Coenzyme Q 10  
COX-2 = Cyclooxygenase-2  
DA = Dopamine  
DAT = Dopamine transporter  
DBS = Deep brain stimulation  
DOPAC = 3,4-dihydroxyphenylacetic acid  
Gpe = Globus pallidus pars externa  
Gpi = Globus pallidus pars interna  
HSP = Heat shock proteins  
IFNγ = Interferon-γ  
IL-1β = Interleukin 1β  
IL-2 = Interleukin 2  
iNOS = Inducible nitric oxide synthase  
LBs = Lewy bodies  
L-dopa = Levodihydroxyphenylalanine  
MAO = Monoamine oxidase  
MPP+ = 1-methyl-4-phenylpyridinium  
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine  
NAC = Non-amyloidogenic component  
NSAID = Non-steroidal anti-inflammatory drugs  
PD = Parkinson’s disease  
PINK1 = PTEN-induced kinase 1  
PLD2 = Phospholipase D2  
RNS = Reactive nitrogen species  
ROS = Reactive oxygen species  
SHSP = Small heat shock protein  
SNc = Substantia nigra pars compact  
STN = Subthalamic nucleus  
TH = Tyrosine hydroxylase  
TNF-α = Tumor necrosis factor-α  
UCHL1 = Ubiquitin-proteasome system  
UPDRS = Unified Parkinson’s Disease Rating Scale  
UPS = Ubiquitin-proteasome system  
VMAT2 = Vesicular monoamine transporter 2  
wild-type = wild-type

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