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REVIEW

Shedding light into the role of BDNF in the pharmacotherapy of Parkinson's disease

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Parkinson's disease (PD) is a chronic, neurodegenerative disease with a 1% incidence in the population over 55 years of age. Movement impairments represent undoubtedly the hallmark of the disorder; however, extensive evidence implicates cognitive deficits as concomitant peculiar features. Brain-derived neurotrophic factor (BDNF) colocalizes with dopamine neurons in the substantia nigra, where dopaminergic cell bodies are located, and it has recently garnered attention as a molecule crucial for cognition, a function that is also compromised in PD patients. Thus, due to its colocalization with dopaminergic neurons and its role in cognition, BDNF might possess a dual role in PD, both as a neuroprotective molecule, since its inhibition leads to loss of nigral dopaminergic neurons, and as a neuromodulator, as its enhanced expression ameliorates cognitive processes. In this review, we discuss the mechanism of action of established as well as novel drugs for PD with a particular emphasis to those interfering with BDNF biosynthesis.
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Aetiology of Parkinson's disease (PD)

PD is a progressive, degenerative disorder characterized by selective loss of nigral dopaminergic neurons, the pathognomic feature of PD, resulting in a pronounced depletion of striatal dopamine, which leads to the most devastating symptoms of the disease, that is, motor dysfunctions. PD is also characterized by decline in cognitive processes, which significantly contribute to the social burden of the disease, suggesting that brain regions other than the nigrostriatal network are affected. Recently, novel insights have been discovered and progress made through identification of other pathological hallmarks of the disease's pathogenesis such as the presence, in the degenerated neurons, of Lewy bodies containing α-synuclein.[1,2] Genetic evidence and environmental influence have also been demonstrated adding to the complexity of this neurodegenerative disorder.

Implication of brain-derived neurotrophic factor (BDNF) in PD: preclinical, clinical and genetic evidence

Among the proteins putatively involved in the pathogenesis of PD, neurotrophic factors play a key role in the neuroprotection of the dopaminergic phenotype.[3] To this regard, the neurotrophin BDNF has been well characterized with respect

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to the interaction with dopaminergic neurons. Several lines of evidence point to a specific role for BDNF in the neuronal degeneration observed in PD. Dopaminergic neurons of the ventral midbrain, substantia nigra and ventral tegmental area express BDNF:[4] the inhibition by antisense oligonucleotide infusion leads to loss of nigral dopaminergic neurons[5] indicating that reduced BDNF mRNA expression in substantia nigra might participate in the death of nigral dopaminergic neurons observed in PD. Baquet *et al*.[6] substantiated this evidence showing that BDNF is required for establishment of the proper number of dopaminergic neurons in the substantia nigra, strenghtening the hypothesis that the degeneration of dopaminergic neurons in PD may be related to reduced BDNF biosynthesis. Interestingly, we have recently shown that BDNF expression is markedly reduced in the frontal cortex of dopamine transporter knockout mice,[7] that are characterized by a massive decrease of striatal dopamine content.[8] Reduced levels of BDNF in frontal cortex may endanger neuronal viability in other structures, such as the striatum, where BDNF is anterogradely transported. As a functional implication, the reduced BDNF levels in frontal cortex may partly explain the striatal neurodegeneration sporadically observed in dopamine transporter knockout mice.[9]

These findings are corroborated by the observation that striatal dopamine output is compromised in BDNF heterozygous mice leading to impaired behavioral responses directly associated with perturbed nigrostriatal dopaminergic system.[10,11] Interestingly, partial deletion of the high-affinity BDNF receptor trkB leads to a reduced number of neurons in the substantia nigra of old mice, which is paralleled by a diminished expression of tyrosine hydroxylase and a marked formation of α-synuclein deposits[12] suggesting that BDNF is critical for the well being of the nigrostriatal dopaminergic neurons during senescence. To this end, Kohno *et al*.[13] showed that pathogenic mutations of α-synuclein can be ascribed to reduced BDNF biosynthesis.

Preclinical evidence is corroborated by clinical reports revealing that nigrostriatal dopamine neurons of PD patients show markedly decreased levels of BDNF[14–17] confirming that reduced amount of the neurotrophin may be involved in the etiology and pathogenesis of PD.

In addition to the above-mentioned evidence, genetic studies have shown potentially functional polymorphisms in the BDNF gene (BDNF val^{66}met polymorphism)[18] that may be associated with higher vulnerability to develop PD, although experimental findings are not unequivocal. In fact several groups demonstrated an association between BDNF val^{66}met polymorphism and PD pointing to the neurotrophin as a candidate gene conferring susceptibility to PD[19–21] whereas other authors did not confirm these findings.[22,23]

Exogenous administration of BDNF: pros and cons

Based on the BDNF deficit observed in PD patients at the nigrostriatal level, the possibility to administer recombinant BDNF in order to preserve dopaminergic neurons and improve symptoms of the disease might well represent a therapeutic opportunity. Ideally, direct replacing of the deficient neurotrophin could solve the problems related to the deprivation of the trophic support to the affected neurons. However, several caveats have hampered so far such a logic approach.

The major obstacle is the large molecular size of the neurotrophin[24,25] that does not readily pass through the blood–brain barrier, leading to a limited diffusion in the brain parenchyma. In addition, it has to be taken into account that, once diffused, the elevated expression of trkB, the high-affinity receptor for BDNF throughout the brain parenchyma may restrict the availability of the neurotrophin to target neurons.[26] Another problem is represented by the inability to site-specifically deliver the neurotrophin into the nigro-striatal system, suggesting that the trophic factor distribution in the target tissue is a key factor for a successful therapy. Researchers have tried to avoid these problems by producing specific carriers that could deliver the neurotrophin inside the cell. Transplantation of immortalized neural progenitor cells that could secrete growth factors[27,28] as well as transfection of viral vectors aimed at delivering BDNF directly into striatum or substantia nigra[29,30] are undoubtedly promising approaches that proved effective in animal models of the disease. Levivier *et al*.[31] have demonstrated that intrastriatal grafts of fibroblasts genetically modified to produce BDNF attenuate the loss of nerve terminals whereas preventing the loss of cell bodies of the rat nigrostriatal dopaminergic pathway caused by 6-OHDA. A similar approach was used by Galpern *et al*.[32] who showed that cell-mediated delivery of BDNF augments dopamine levels in the MPP^+ rat model of PD. Furthermore, intrathecal administration of the neurotrophin reduces symptoms of PD in monkeys.[33] Recently, gelling hydrogels providing local delivery of BDNF in the injuried spinal cord have been created.[34] Although different biomaterials can act as scaffolds for the delivery of the neurotrophin, to date evidence of successful results in humans are poor,[35] mainly because their use is limited by many variables, such as the small number of individuals available for clinical trials, difficulty in establishing inclusion criteria and lack of control patients.

Additional problems may be represented by the duration of exogenous BDNF treatment (the length of the treatment necessary to provide protection is unknown), by the rate of BDNF delivery (in order to avoid inactivating enzymes, such as proteases, to degrade the neurotrophin) or by the pharmacokinetic of BDNF itself, that could be different in various brain regions.

Based on these data, protection of dopaminergic neurons from neurodegeneration may be achieved only if exogenous BDNF administration fullfills several requirements, ie an effective dosing regimen provided in an optimal timed and localized manner. If any of these properties is not observed, then external BDNF supply might result ineffective or even more harmful than helpful. To this end, mice overexpressing BDNF show spontaneous seizures[36] and transgenic mice

with increased trkB signaling cascade display increased severity of status epilepticus,[37,38] confirming that miscalibration of BDNF expression levels can lead to severe and undesirable side effects.

An alternative strategy to overcome these problems could be the modulation of endogenous BDNF in those brain regions where the neurotrophin expression is deficient. In this manuscript, we revise the data in support of the possibility to modulate endogenous BDNF expression in dopaminergic neurons as a way to retard the onset of the disease or attenuate the neuronal degeneration, focusing on drugs in preclinical and clinical development.

Dopaminergic drugs

Dopamine deficiency in the nigro-striatal pathway is indeed the leading consequence of neuronal degeneration in PD. For this reason, the most effective therapies, although symptomatic, target selectively the dopaminergic system following different strategies, that is, increasing dopamine biosynthesis (L-DOPA), decreasing dopamine degradation (MAO-B inhibitors such as selegiline), stimulating remaining dopaminergic receptors (pramipexole, bromocriptine). Essentially, these drugs, beyond replenishing dopamine, may contribute to preserve integrity and vitality of dopaminergic neurons: such goal may be reached, at least in part, through modulation of BDNF expression. In fact the regulation of the neurotrophin biosynthesis is indeed a common step in the pro-regenerative properties elicited by such drugs. In particular, L-DOPA enhances striatal expression of BDNF in healthy mice[39] but also it may act, at least in part, through BDNF-induced augmentation of D3 receptor synthesis[40] whose density is reduced in PD patients.[41] It is important to take into account that BDNF mRNA is not synthesized in striatum but it is transported from the cortex:[42] in line with the effect of L-DOPA in PD the drug increased BDNF expression in frontal cortex following either acute or chronic treatment.[40]

An alternative option in the treatment of PD is to allow dopamine to last longer in the synaptic cleft in order to stimulate the remaining dopamine receptors. This may be achieved, for example, by using selective monoamine oxidase inhibitors such as selegiline or rasagiline. '*In vitro*' studies have shown that both drugs increase the expression of BDNF leading to the hypothesis that their protective effect might, at least in part, be driven by the neurotrophin upregulation.[43] Recent studies have mainly focused on rasagiline. Rasagiline is effective as monotherapy or in combination with L-DOPA in early and late PD.[44] A recent manuscript demonstrated that rasagiline increased the expression of different neurotrophic factors, namely BDNF, NGF and glial cell line-derived neurotrophic factor (GDNF) suggesting that the up-regulation of these genes may be neuroprotective and ameliorate cognitive processes.[45] This mechanism of action is shared by the bifunctional drug ladostigil (TV3326), which is a combination of the MAO inhibitor rasagiline with cholinesterase inhibitors, a singular entity that combines different activities and may synergistically offer neuroprotective and cognitive enhancing effects via a simplified drug regimen (Youdim and Buccafusco[46]). Such a combination has been found protective in a model of MPTP-induced neurotoxicity[47] and may also result effective in animal models of Alzheimer's disease.[48]

Stimulation of remaining dopamine receptors represents a further therapeutic approach. To this regard, the D3 receptor-preferring agonist pramipexole, an appropriate choice for initial treatment of PD,[49,50] was found neuroprotective in the MPTP animal model of PD[51] at clinically suitable dosing regimen, as measured by recovery of striatal TH- and DAT immunoreactivity that was significantly reduced in MPTP-treated mice. Presgraves *et al*.[52] suggest that, *in vitro*, the neuroprotective action of pramipexole is mediated by BDNF since antibodies against the neurotrophin blocked the protection afforded by the D3 agonist.

To sum up, the different strategies herein illustrated point to the preservation of the integrity of remaining dopaminergic neurons, at least in animal models of the disease, and share the common property to upregulate BDNF expression, further indicating that modulation of the neurotrophin may help in preventing or slowing down neuronal degeneration. However, we have to consider that other neurotrophic factors can account for the neuroprotective action of the agents mentioned above. For instance, GDNF is a neurotrophic factor with restorative effects in rodent and primate models of PD.[53] In addition, we have shown that the dopamine D2 agonist quinpirole enhances the expression of basic fibroblast growth factor (FGF-2),[54] a trophic factor whose action is essential for the survival of dopaminergic neurons.[55,56] Thus, drugs used against PD might also regulate other trophic factors, probably affording a higher degree of neuroprotection through a combined action.

Glutamatergic drugs

Interaction between glutamate and BDNF plays an important role in regulating the activity of dopaminergic neurons in the substantia nigra[57] suggesting that modulation of glutamatergic systems may be therapeutically relevant for PD. Antagonists of the NMDA receptors may be useful in the pharmacological therapy of PD in an attempt to mitigate the imbalance between dopaminergic and glutamatergic pathways in the basal ganglia. To this regard, memantine, an uncompetitive NMDA receptor antagonist, has been used for the treatment of PD with positive results.[58,59] Blockade of NMDAergic neurotransmission, as provided by memantine, may prove relevant to counteract the excitotoxicity produced by such imbalance and concomitantly may improve cognitive symptoms via BDNF modulation.[60]

Besides NMDA receptors, modulation of AMPA receptors may represent an opportunity for PD, as previously suggested for AD. Among different AMPA potentiators, LY503430 provides dose-dependent functional neuroprotection in rodent models of PD.[61] When used following MPTP administration or 6-OH-dopamine infusion, LY503430 was

able to reduce loss of tyrosine hydroxylase striatal immunoreactivity as well as to correct apomorphine-induced rotational asimmetry,[62] with concomitant induction of BDNF expression in the substantia nigra. Based on these findings, it is conceivable to hypothesize that AMPA potentiators would protect against nigrostriatal degeneration, at least in part, through BDNF upregulation.

In addition to ionotropic receptors, metabotropic glutamate receptors (mGluRs) may represent a valuable target for pharmacotherapy of PD, in particular mGluRII. These receptors are located presynaptically in the subthalamic nuclei and substantia nigra suggesting that their activation would reduce glutamate release from these brain areas resulting in a diminished hyperactivity of the subthalamic nuclei, a pathophysiological feature of PD.[63] Another rationale for their therapeutic use implies that mGluRII receptors could stimulate dopamine release from surviving dopaminergic neurons in rat substantia nigra.[64] In line with these theories, Matarredona *et al*.[65] have demonstrated that mGluRII activation protects striatal dopaminergic nerve terminals against MPP^+-induced neurotoxicity along with BDNF induction.[65] The protective effects of mGluRII activation are not limited to experimental Parkinsonism but include protection against ischemia,[66] excitotoxic damage[67] and nitric oxide-induced programmed cell death.[68]

Taken together, it is possible to suggest that the modulation of glutamatergic systems might be of clinical relevance by virtue, at least in part, of increased BDNF production implying that the neurotrophin might actively participate in affording neuroprotection in PD.

Nicotine

The rationale of a nicotine-based approach for PD derives from epidemiological studies showing a reduction of the risk and the incidence of PD in smokers compared to non-smokers,[69–74] although some inconsistencies have been demonstrated.[75] Interestingly, PD patients seem to smoke at a lower rate than non-affected individuals.[76]

Among the widespread pharmacological effects exhibited by nicotine, it is known that its effects are mediated by the stimulation of acetylcholine-gated ion channel receptors.[77] The relationship between nicotine and PD is justified by the evidence that nicotine activates damaged dopaminergic neurons in striatum;[78] the nicotine-dependent dopamine release might be relevant in PD since it could mitigate motor symptoms as well as protect against different types of insults in experimental animal models of the disease. To this end Schneider *et al*.[79,80] have shown that the combination of low, ineffective doses of both the neuronal nicotinic $\alpha4\beta2$ agonist SIB-1508Y and L-DOPA significantly attenuated motor and cognitive dysfunctions in parkinsonian monkeys suggesting that administration of subtype-selective nicotinic acetylcholine receptor agonists may help reduce L-DOPA doses that are necessary to produce a therapeutic effect[79] thus revealing a potential usefulness as adjunctive therapy. In addition, nigrostriatal damage could be mitigated by nicotine-driven dopamine release. For example, in the MPTP model of PD, enhanced dopamine in the synaptic cleft might compete with MPP^+ thus attenuating the neurotoxicity brought about by the toxin.[81] Alternatively, nicotine may exert neuroprotectant activity through other mechanisms, for example by inducing BDNF expression in different brain areas. To this regard, we have shown the neuroprotective effects of nicotine in two models of PD, that is, MPTP- and methamphetamine-treated rats, by virtue of increased BDNF expression, thus implying modulation of the neurotrophin as a mechanism through which nicotine protects from experimental parkinsonism.[82] Support to this theory derives from French *et al*.[83] as well as Kenny *et al*.[84] who showed increased expression of hippocampal BDNF following both acute and chronic nicotine administration raising the possibility that nicotine might improve cognition, at least in part, by such a mechanism. These findings raise the interesting issue that nAChR-based pharmacological treatments may help the therapy of cognitive dysfunctions associated with PD.[85–87]

Immunophilins

An innovative approach in the treatment of PD is represented by neuroimmunophilins. Immunophilins are the receptors for immunosoppressive drugs. These compounds represent a relatively new class of drugs with demonstrated effectiveness in animal models of PD.[88–90] Tanaka and associates[91] have evaluated the ability of immunosoppressive (FK506) and non-immunosoppressive (GPI1046) drugs to activate neurotrophic factors such as BDNF and GDNF. Whereas, both compounds were able to enhance GDNF expression, only the immunosoppressive molecule induced striatal expression of BDNF. Although immunosoppressant activity is indeed important for different purposes, immunophilin ligands devoid of immunosoppressant properties would be fascinating therapeutic tools for neurodegenerative disorders. To this regard, Nitta *et al*.[92] have synthesized a hydrophobic dipeptide (Leu-Ile) that partially resembles the site on FK506 that binds to immunophilin. Leu-Ile increased BDNF expression in cultured hippocampal neurons as well as in mesencephalic cell cultures that were protected by the dipeptide against neuronal cell death. Interestingly, when mesencephalic cell cultures derived from BDNF knockout mice were employed, Leu-Ile was not able to afford neuroprotection.[92] *In vitro* results were confirmed by subchronic (5 days) treatments with the dipeptide which resulted in a significant increase of striatal BDNF levels.[92]

These observations are indeed interesting in view of the clinical trials with different synthetic immunophilins currently under way[93] and further point to BDNF as one of the pivotal targets through which neuroprotective compounds might promote their activity. The first clinical trial (6-month treatment with a neuroimmunophilin ligand called GPI-1485) did not yield significant results. Longer clinical trials (2 years) are currently underway to investigate whether a prolonged administration of these compounds

may be beneficial.[93] It is important to note that these compounds, or their derivatives, improve spatial memory and reverse cholinergic atrophy in aged mice[94] suggesting that they might also represent an alternative strategy for AD.

Herbal extracts

In close relationship with the immunosoppressant FK506, it has been found that the immunosoppressive extract Tripchlorolide (designated as TW397) exerts neuroprotective properties and protects dopaminergic neurons in the MPTP model of PD.[95] In addition, repeated administration of TW397 attenuated the rotational behavior caused by amphetamine administration in rats with the lesion of the medial forebrain bundle (MBF), promoted the survival of subtantia nigra pars compacta as well as prevented the dopamine decrease in the striatum of MBF-lesioned rats.[95] These findings suggest a potential therapeutic value for TW397 in PD. *In vitro* studies revealed that the molecular mechanism supposed to be responsible for the neuroprotection afforded by this compound seems to rely on increased BDNF expression. In fact, TW397 at the concentrations optimal for inducing axonal elongation significantly increased BDNF expression indicating that this herbal extract might represent an attractive compound to be tested in clinical trials.[95]

Erythropoietin

Recently a huge emphasis has been put on the natural hormone EPO, produced by the adult kidney, that has been used for more than a decade for treatment of anemia. EPO induces its biological effects through the interaction with the specific receptor whose expression is detectable in the central nervous system throughout the entire life.[96] Detailed studies in recent years have clearly suggested that EPO is neuroprotective in a wide variety of animal models ranging from acute diseases, such as traumatic brain injuries[97] or spinal cord lesions,[98] to chronic neurodegenerative disorders. To this regard, EPO has been shown to ameliorate the latency and severity of seizures induced by kainate[99] and to improve neurological function recovery in experimental autoimmune encephalomyelitis.[100]

Preclinical studies have demonstrated that, among the intracellular modifications brought about by EPO, this cytokine regulates the expression of BDNF under different experimental paradigms.[100,101] EPO is also effective in animal models of PD. In fact, in MPTP-treated rats, a decrease in TH-positive neurons was completely reverted by administration of EPO.[102] In the same animal model, EPO restores brain antioxidant and glutathion peroxidase activity in both striatum and substantia nigra.[103]

The effects of EPO on BDNF expression has been recently strengthened by Viviani *et al*.[104] who demonstrated that both '*in vitro*' and '*in vivo*' EPO-induced neuroprotection is mediated by BDNF. These authors have shown that, in hippocampal neurons, EPO administration rescues as many as 50% of neurons from death caused by the neurotoxicant trimethyltin: such effect was directly linked to increased BDNF expression since the use of an anti-BDNF antibody abrogated the neuroprotective effect.[104] They also showed that '*in vivo*' the neuroprotective activity provided by EPO is BDNF dependent since intracerebroventricular injection of the cytokine significantly enhances the neurotrophin expression. Taken together, these observations undoubtedly point to BDNF as the major mediator of EPO-induced neuroprotection.

The possibility to develop EPO-like compounds devoid of the hematopoietic effects is, though, a fundamental step since clinical use of EPO in the treatment of chronic neurodegenerative disorders is precluded because of these undesirable effects.

Cannabinoids

As a further strategy that might be employed to prevent or combat PD, cannabinoids represent a very recent option. These substances are neuroprotective against excitotoxicity, hypoxia and cerebral ischemia[105,106] and are effective in animal models of Alzheimer's disease.[107] Previous studies had suggested that cannabinoids might be therapeutically relevant in PD either by mitigating motor symptoms[108] or by reducing levodopa-induced dyskinesia.[109] Lastres-Becker *et al*.[110] have demonstrated that Δ9-tetrahydrocannabinol, administered for 2 weeks, abolishes reductions in dopamine contents and TH immunoreactivity produced by 6-hydroxydopamine, thus counteracting the neurodegeneration caused by the neurotoxin. Interestingly, the same neuroprotectant properties have been shown by cannabidiol, a plant-derived cannabinoid with negligible affinity for cannabinoid CB1 receptors, suggesting that cannabinoids, through independent receptors, might afford neuroprotection in an animal model of PD. These neuroprotective actions were also shown in cultures of mouse cerebellar granule cells.[110] The recent observation from Butovsky *et al*.[111] showing marked upregulation of BDNF in different brain regions of animals chronically treated with Δ9-tetrahydrocannabinol suggests that the induced neuroprotection in animal models of PD might depend, at least in part, upon the neurotrophin upregulation.

Lifestyle

PD is a neurodegenerative disease with a complex epidemiology, that may depend also on environmental conditions. Environment has a dual role: it may orient brain cells toward higher susceptibility to PD (for example exposure to neurotoxins or use of high-calorie, high-fat diets[112]) as well as it might provide neuroprotection against PD (for instance exercise[113–115] or controlled diet[116]).

Thus, the emerging view suggests the possibility to interfere with the course of the disease through environmental manipulations. Although it is hard to establish the mechanisms through which environmental manipulations

might positively affect the course of PD, several lines of evidence point to the 'indirect' contribution of BDNF.

Enriched environment has been shown to confer resistance to MPTP-induced parkinsonism in mice by preventing neurodegeneration through an attenuated loss of dopamine neurons.[117,118] Interestingly, mice exposed to enriched environment showed an increase in BDNF expression raising the possibility that environment might mitigate neuronal loss through BDNF upregulation.[117,118]

The role of physical activity in preventing PD onset or progression has also been demonstrated. It is possible to speculate that such prevention could be obtained, at least in part, by means of increased BDNF expression since voluntary exercise enhances the neurotrophin expression in different rat brain regions including hippocampus, cortex and striatum.[119–121] However, Poulton and Muir[122] have demonstrated that treadmill training does improve dopamine loss but not behavioral deficits in 6-OH-DA-treated rats suggesting that many factors can interact with exercise to determine the effectiveness of such therapy.

Recent data have shown that caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of PD,[116] suggesting that dietary restriction affects the course of the disease by altering the synthesis of trophic factors. Attention has been focused on the effects of dietary Vitamin E intake and PD, although results from clinical trials have produced contrasting results (Parkinson's Study group, 1993;[123]). Interestingly, Vitamin E has been shown to counteract the deleterious effect of a saturated fat diet through BDNF normalization, thereby improving synaptic plasticity and cognition.[124] These results suggest that diet may interfere with neuronal and behavioral plasticity, through modulation of BDNF.

Taken as a whole, it appears that environmental stimuli of different types, although distinct in nature, can influence the onset, severity and course of the disease, at least in part by modulating BDNF expression. These data emphasize the need to consider environmental influence as an important etiological factor for PD suggesting that drugs mimicking the protective effect of environmental stimulation may prove beneficial for PD patients.

Conclusion

From the evidence mentioned in this manuscript, it could be inferred that the dual role of BDNF both as a neuroprotectant or neuromodulator of dopaminergic function in the central nervous system. On one side the neurotrophin is essential for maintaining healthy neurons in the nigrostriatal circuitation thus preventing movement deficits to take place while, on the other side, it actively participates to activity-dependent processes such as synaptic plasticity and cognition that are compromised in PD. Such dual role is strictly related to the regional specificity of the neurotrophin change that might dictate the nature of the role played by BDNF. In the substantia nigra, BDNF is essential for survival of dopaminergic neurons under physiological conditions and has found to be decreased in PD patients: increased BDNF expression might thus provide trophic support to nigral dopaminergic-degenerating neurons thus preventing their loss. In frontal/prefrontal cortex and hippocampus, neuroprotection afforded by BDNF might attenuate the cognitive decline observed in PD patients.

Since BDNF has been shown to be a prerequisite of well being for the dopaminergic neuron, preventive non-invasive 'therapy' by means of a correct lifestyle, including a balanced diet, good level of physical activity and an interactive social life, may result critical in preventing the onset or attenuating the severity of the neurodegenerative process. The combination of pharmacologic treatment with a proper lifestyle might synergize in the modulation of BDNF. Although speculative and not supported by experimental data, such a combinatorial approach offers a new opportunity that could optimize the outcome of therapeutic interventions.

We have to take into account that current and proposed therapies may also exert, by different mechanisms, a more global neurotrophic action, for instance a general enhancement of synaptic plasticity. To this regard, by different means, BDNF, NGF, GDNF and FGF-2 are intimately linked to synaptic plasticity and rearrangement of brain cytoarchitecture. Thus, the possibility to trigger a coordinated and spatial upregulation of different trophic factors may positively impact synaptic plasticity in the Parkinsonian brain.

Furthermore, it has been demonstrated that, following 6-OH-DA lesion of the nigrostriatal pathway, the phosphorylation of striatal proteins, such as CaMKII alpha and DARPP-32, is significantly altered, an effect that is completely reversed by L-DOPA treatment.[125] Given the crosstalk existing between BDNF- with CaMKII alpha- and DARPP-32-dependent pathways,[126–128] it is conceivable to hypothesize that modulation of BDNF by drug therapy may initiate a cascade of mutually regulating signals resulting in an overall enhancement of synaptic plasticity.

Indeed therapeutic interventions for PD will involve the modulation of multiple proteins or intracellular mechanisms. To this end, we have to take into account that the trophic requirement for dopaminergic neurons is not afforded by BDNF alone. A very recent manuscript showed that GDNF performed better than BDNF for protecting nigrostriatal dopaminergic neurons and correcting the related behavioral deficits caused by intrastriatal injections of 6-hydroxydopamine.[129] However, the capability to restore dopaminergic neurons was not additive or synergistic when the two neurotrophins were co-expressed.[129] This evidence is corroborated by Onyango *et al*.[130] who demonstrated that BDNF and GDNF utilize distinct intracellular signaling pathways to protect cytoplasmic hybrid cells made from mitochondrial DNA of idiopathic PD individuals. These observations add to the complexity of the mechanisms responsible for the neurotrophin-mediated neuroprotection of dopaminergic neurons and further pinpoint to the selective modulation of endogenous trophic factors as a feasible option to correct specific deficits of the disease.

Furthermore, the possibility to therapeutically target the high-affinity receptor of BDNF, trkB, should also be taken into account.[131] For example, it has been shown that trkB mRNA levels are possibly related to antidepressant treatment[132,133] suggesting that trkB is sensitive to amine modulation. In addition, decreased phosphorylation levels of trkB was observed in neurodegenerative disorders[134] suggesting that normalization of the intracellular signaling mediated by the BDNF receptor might be therapeutically relevant.

Although the development of BDNF-mimicking drugs is indeed premature, the identification of additional targets for pharmacotherapy may prove relevant in improving the therapeutic armamentarium for PD. Based on the finding that BDNF expression in dopaminergic neurons tightly correlates with their correct functioning, the evidence reported in this review suggests that dysregulation of BDNF is a pathophysiological mechanism of the disease and point to BDNF as a putative target of new therapeutic interventions aimed at preventing or restoring the ongoing neurodegenerative processes. Thus, BDNF may be viewed as itself therapeutic and target of therapeutics; however, due to the difficulties of administering exogenous BDNF and the related paucity of data in humans, the endogenous modulation of BDNF may represent an additional, helpful property of drugs used to fight PD. Furthermore, although it might be considered a bit speculative, the evaluation of endogenous BDNF modulation could be considered a criteria, among others, to screen new therapeutic interventions for this devastating disorder.

Thus, in conclusion, BDNF appears to play a multifaceted role in PD since it could be viewed as a diagnostic and preventive tool as well as, perhaps, a target for more specific treatments.

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