New Synaptic and Molecular Targets for Neuroprotection in Parkinson's Disease

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ABSTRACT: The defining anatomical feature of Parkinson's disease (PD) is the degeneration of substantia nigra pars compacta (SNc) neurons, resulting in striatal dopamine (DA) deficiency and in the subsequent alteration of basal ganglia physiology. Treatments targeting the dopaminergic system alleviate PD symptoms but are not able to slow the neurodegenerative process that underlies PD progression. The nucleus striatum comprises a complex network of projecting neurons and interneurons that integrates different neural signals to modulate the activity of the basal ganglia circuitry. In this review we describe new potential molecular and synaptic striatal targets for the development of both

symptomatic and neuroprotective strategies for PD. In particular, we focus on the interaction between adenosine A2A receptors and dopamine D2 receptors, on the role of a correct assembly of NMDA receptors, and on the sGC/cGMP/PKG pathway. Moreover, we also discuss the possibility to target the cell death program parthanatos and the kinase LRRK2 in order to develop new putative neuroprotective agents for PD acting on dopaminergic nigral neurons as well as on other basal ganglia structures. © 2012 Movement Disorder Society

Key Words: synaptic plasticity; dopamine receptors; NMDA receptors; LRRK2, Parthanatos

The main anatomical feature of Parkinson's disease (PD) is the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc), resulting in the loss of endogenous dopamine (DA) in the striatum. The DA precursor L-dopa and other antiparkinsonian medications partially alleviate striatal DA deficiency. However, after years of treatment, patients become less responsive to L-dopa as the disease progresses and experience dyskinesias.¹ In addition to dopaminergic degeneration, PD also results in the loss of nondopa-

minergic neurons throughout the nervous system.² For this reason preclinical and clinical neuroprotective studies have been focused on protecting either dopaminergic neurons projecting to the striatum or nondopaminergic neurons critically involved in the pathophysiology of the disease.³

Maintenance of the correct functional assembly of synaptic striatal circuits and preservation of the fine striatal spine morphology of medium spiny neurons (MSNs) have been considered a secondary target in neuroprotection studies. The maintenance of this physiological equilibrium is a direct consequence of the restorative dopaminergic strategies. It has been assumed that striatal MSNs were free of pathology until the late phase of the disease, when they show progressive loss of dendritic spines.⁴ Striatal spine morphology and function are critically important because the "head" of striatal spines receives excitatory glutamatergic projections from the cerebral cortex, whereas dopaminergic projections from the SNc contact on the "neck" of the spines. In addition, glutamatergic projections from specific thalamic nuclei terminate on the dendritic shafts. The size of the dendritic trees of

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MSNs is significantly reduced in the caudate nucleus of brains of PD cases, and their complexity is changed in both the caudate nucleus and the putamen. Considering that dendritic spines integrate glutamatergic excitatory inputs and dopaminergic signals, reduction in both the density of spines and total dendritic length is likely to have a dramatic impact on the ability of these neurons to properly function and may partly explain the symptoms of the disorder.^{4,5} One of the reasons for this corticostriatal dysfunction might be represented by the Lewy body pathology appearing in the pyramidal neurons of the deep cortical layers (V–VI), where the cell bodies of the corticostriatal projections are located.⁶ Interestingly, a multiphoton imaging approach has revealed that after striatal denervation, striatopallidal MSNs are mainly involved in this process of loss of spines and glutamatergic synapses via a mechanism requiring dysregulation of Cav1.3 L-type Ca^{2+} channels at the level of the spines.⁷

Three-dimensional electron microscopy of tissue from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys shows that striatal spines contacted by cortical terminals display an increased volume of their spine apparatus, suggesting increased protein synthesis at corticostriatal synapses. Thalamostriatal and corticostriatal afferents target different types of striatal spines, and these systems undergo partly different ultrastructural changes. Nevertheless, in both systems, ultrastructural changes are indicative of increased strength of glutamatergic striatal transmission in this model of PD.⁸

New ultrastructural findings also suggest that astrocytes are integral functional elements of tripartite glutamatergic synaptic complexes in the cerebral cortex and the striatum. In the striatum of parkinsonian animals there is significant expansion of the astrocytic coverage of tripartite synapses, suggesting ultrastructural compensatory changes that affect both neuronal and glial elements. These findings suggest that new neuroprotective strategies have to take into account the concept of glial–neuronal communication in the striatum, where astrocytes respond to changes in neuronal activity regulating extracellular transmitter levels and excitatory transmission.⁹

A2A Adenosine/D2 Dopamine Receptors: From Synaptic Effects to Neuroprotection

Adenosine receptors are G protein–coupled receptors (GPCRs) that mediate the physiological functions of adenosine. In the central nervous system adenosine A2A receptors (A2ARs) are selectively expressed in the striatum, where they are mainly located postsynaptically in D2 DA receptor–expressing striatopallidal

MSNs.^{10,11} In striatopallidal neurons they form functional oligomeric complexes with other GPCRs including D2 receptors. Blockade of A2A receptors with specific antagonists facilitates striatal D2 receptor function. Thus, A2AR antagonists can be considered prodopaminergic agents and could potentially reduce the effects associated with DA depletion in PD. This class of compounds has recently attracted considerable attention for PD pharmacotherapy for counteracting motor dysfunction. Moreover, A2AR antagonists are also neuroprotective in animal models of PD.^{12–14} In PD patients with L-dopa-induced dyskinesia, there is increased striatal A2A receptor availability, suggesting altered adenosine transmission in dyskinesia and providing an additional rationale for testing A2A receptor antagonists in this motor complication.^{15,16}

Epidemiological and preclinical data suggest that caffeine may confer neuroprotection against the underlying dopaminergic neuron degeneration and may influence the onset and progression of PD. It has been demonstrated that among the various pharmacological effects attributed to caffeine, this agent exerts A2A receptor antagonistic properties. Thus, it is possible that caffeine and its derivatives via the modulation of A2A receptors might reduce PD symptoms and possibly exert neuroprotective effects.¹⁷

Molecular and electrophysiological data suggest a direct interaction between A2A receptors and the receptor tyrosine kinase fibroblast growth factor receptor (FGFR).¹⁸ Concomitant activation of these 2 receptors, but not individual activation of either alone, causes robust activation of the MAPK/ERK pathway, differentiation and neurite extension of PC12 cells, and spine morphogenesis in primary neuronal cultures. Interestingly, a novel interaction between A2A and FGFR receptors also controls corticostriatal plasticity.¹⁸ The demonstration of synergy between the A2A and FGFR receptors might have important clinical implications considering the localization of A2A receptors in striatopallidal neurons, where they influence the actions of D2 receptors. Accordingly, the neuroprotective effects of basic FGF in focal brain ischemia have been demonstrated.¹⁹ Thus, the discovery of cross talk between the A2A and the FGFR opens new possibilities for therapeutic interventions directly targeting striatal circuitry dynamics.

A2A and D2 receptors converge in the control of corticostriatal glutamatergic transmission by exerting an opposite function.²⁰ The possible molecular mechanisms underlying this interaction and the neuronal subtypes implicated in this negative modulation of glutamate release have recently been investigated.²¹ This study suggested that endocannabinoid and cholinergic interneurons take part in this modulatory function (Fig. 1).

Recently the effect of subchronic blockade of A2A receptors on striatal neuron physiology and

FIG. 1. Striatal D2/A2A interaction on cholinergic interneurons modulates both D1- and D2-receptor-expressing medium spiny neuron (MSN) activity. In the nucleus striatum activation of both dopamine D2 and adenosine A2A receptors decreases the activation of cholinergic interneurons and the release of acetylcholine. The subsequent lowering of the M1 muscarinic receptor tone favors the disinhibition of Cav1.3 Ca^{2+} channels, the increase in intracellular calcium concentration, and finally the production and release of endocannabinoids (ECBs). ECBs travel across the synapse and activate presynaptic CB1 receptors, causing reduced glutamate release, thus modulating excitatory synaptic transmission onto both (left) D1- and (right) D2-receptor-expressing medium spiny neurons (ACh, acetylcholine; DA, dopamine; Glu, glutamate; GluRs, glutamate receptors).

morphology has been addressed in a murine model of PD.²² The A2A receptor antagonist SCH58261, systemically administered to DA-depleted mice for 5 days, significantly attenuated alterations in synaptic currents, spine morphology, and dendritic excitability of MSNs of the indirect pathway caused by DA depletion. However, this treatment failed to prevent the spine loss observed after denervation in this subtype of MSNs. This study suggests that subchronic A2A receptor antagonism does not fully prevent ultrastructural striatal spine alterations, but it improves striatal function by attenuating synaptic adaptations of MSNs to DA depletion.²²

The interaction between A2A receptors and dysfunctional mitochondrial activity in PD represents another interesting target for the identification of new neuroprotective strategies for PD. Complex I is the first protein component of the mitochondrial respiratory chain, and it plays a crucial role in ATP production and mitochondrial function. Mitochondrial dysfunction has been identified in a number of neurodegenerative diseases, in particular in PD. Mitochondrial toxins such as rotenone and MPTP are capable of producing relatively selective neuronal cell death and have been used to produce models of PD.^{23,24} The possible neuroprotective action of A2A receptor antagonists against mitochondrial dysfunction has been investigated in striatal slices exposed to the acute application of rotenone, a pesti-

cide acting as a selective inhibitor of mitochondrial complex I activity.²⁵ In striatal slices rotenone causes irreversible loss of corticostriatal field potential amplitude as well as membrane depolarization. A2A receptor antagonists reduced the irreversible electrophysiological effects induced by this mitochondrial toxin, suggesting that the treatment with A2A receptor antagonists might represent a possible neuroprotective strategy in basal ganglia disorders involving a deficit of mitochondrial complex I activity such as PD.²⁵

NMDA Receptor Subunit Composition and Its Role in Physiological and Aberrant Striatal Synaptic Plasticity as a Possible Target for Neuroprotection

NMDA receptors have been implicated in the induction of the major forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD). These forms of plasticity are believed to underlie complex tasks including learning and memory (Kandel, 2001). NMDA receptors also play a critical role in striatal LTP, which is considered a synaptic and cellular model for motor learning.^{26–29} Moreover, NMDA-mediated aberrant striatal synaptic plasticity has been implicated in several experimental models of neurological diseases including PD,^{30,31} L-dopa-induced dyskinesia,^{1,32,33} Huntington's disease,^{34,35} epilepsy,^{36,37} ischemia,^{38–40} and Down syndrome.⁴¹ Alterations of the NMDA receptor channel complex have been identified as an underlying molecular mechanism in neurological disorders ranging from acute stroke to chronic neurodegeneration. Excessive glutamate levels have been detected in pathological conditions including PD, resulting in abnormal stimulation of NMDA receptors, which in turn trigger a cascade of excitation-mediated neuronal damage and apoptosis via Ca^{2+} -dependent mechanisms. Thus, NMDA receptors have been identified as potential targets for neuroprotective strategies. Although competitive NMDA receptor antagonists have been found to be neuroprotective in animal models, these drugs failed in clinical trials because of serious adverse effects such as cognitive alterations and sedation.⁴²

Recently, the concept that the pathways' downstream NMDA receptor activation could represent a key variable element among neurological disorders has been suggested.⁴³ In particular, variations in NMDA receptor subunit composition could be important in neurodegenerative processes. The possibility to target specific NMDA subunits rather than to use competitive NMDA antagonists has been explored as a symptomatic as well as neuroprotective strategy. The NMDA receptor complex has been shown to be altered in experimental models of parkinsonism and in

PD in humans. Changes of specific NMDA receptor subunits as well as postsynaptic associated signaling complex including kinases and scaffolding proteins occur in experimental parkinsonism and PD.⁴³ These findings may allow the identification of specific molecular targets whose pharmacological or genetic manipulation might lead to innovative therapies for PD. The NR1 subunit and PSD-95 protein levels are selectively reduced in the postsynaptic density (PSD) of fully DA-denervated striata. These effects are accompanied by an increase in striatal levels of α CaMKII autophosphorylation coupled with higher recruitment of activated CaMKII to the regulatory NMDA receptor NR2A-NR2B subunits. The effect of DA denervation was mimicked by the blockade of D1-like but not D2-like receptors. Administration of CaMKII inhibitors reversed both the alterations in corticostriatal synaptic plasticity and the deficits in spontaneous motor behavior found in this model of PD. A similar beneficial effect was obtained following subacute L-dopa, suggesting that normalization of CaMKII activity may represent a critical mechanism of the therapeutic effect of L-dopa in PD.⁴⁴

Full DA denervation in experimental animal models mimics advanced PD, causing altered plasticity,²⁷ loss of striatal dendritic spines,^{7,45} and changes of glutamatergic signaling.^{44,46} After complete dopaminergic denervation, there is loss of both LTP and LTD at striatal glutamatergic synapses, indicating that the integrity of the nigrostriatal dopaminergic pathway is of crucial importance for the induction of striatal long-lasting synaptic changes.^{28,31,47–50} However, it is important to stress that early clinical symptoms of PD are only detected when >70% of DA neurons are lost.⁵¹ For this reason, a reliable model of early PD characterized by lowered DA close to this threshold is of key importance in understanding the molecular and synaptic changes underlying this early stage and finding new therapeutic strategies to treat early phases of the disease. Following this line of research, it has been recently shown that distinct levels of DA denervation differentially alter the induction and maintenance of 2 distinct and opposite forms of corticostriatal synaptic plasticity.⁵² Partial DA denervation does not affect corticostriatal LTD. However, this form of synaptic plasticity is blocked following full denervation, indicating that a low, although critical, level of endogenous DA is necessary for LTD. This observation leads to the hypothesis that LTD is implicated in the motor abnormalities seen in the late phase of PD but not in the early stage of the disease. Conversely, partial DA denervation affects the maintenance of LTP, confirming the critical role of this form of synaptic plasticity in early motor parkinsonian symptoms.⁵²

Previous reports have clearly addressed the capability of cell-permeable TAT peptides fused to the C-terminal domain of NMDA receptor subunits to

reach and to disrupt NMDA/MAGUK association in both *in vitro* and *in vivo* studies.^{53,54} Exposure to a TAT peptide fused to the last 9 C-terminal amino acids of NR2A (TAT2A)⁵³ directly targets PSD-95–NR2A subunit interaction and can reverse motor and synaptic plasticity abnormalities in early parkinsonism. Systemic administration of TAT2A peptide normalizes both LTP and motor behavior in partially lesioned rats, which could be considered a model of “early” PD (Fig. 2). The possibility of targeting intracellular pathways and protein complexes using cell-permeable peptide conjugates represents a possible new and potent approach to blocking intracellular pathways implicated in neurodegenerative processes with reduction of the side effects related to the direct antagonism of the NMDA receptor channel complex.⁵⁵

The NO–sGC–cGMP Signaling Pathway in Striatal Plasticity and Neuroprotection

Nitric oxide (NO) is a diffusible gaseous molecular agent of critical importance in both physiological and pathological functioning of brain activities, acting either as a retrograde transmitter or as a mediator of toxicity. In addition to its role in neurotransmission of central and peripheral neurons, NO mediates blood vessel relaxation by the endothelium and immune activity of macrophages. NO is produced from 3 NO synthase (NOS) isoforms: Neuronal NOS (nNOS), endothelial NOS, and inducible NOS (iNOS). Excessive production of NO following a pathologic insult can lead to neurotoxicity. NO plays a role in mediating neurotoxicity associated with a variety of neurologic disorders, including stroke, PD, and HIV dementia.⁵⁶

In the striatum NO is produced by interneurons⁵⁷ and it plays an important role in the regulation of corticostriatal synaptic plasticity⁵⁸ and motor activity.⁵⁹ Concomitant activation of NMDA and D1-like DA receptors in the striatum triggers NO synthesis.⁶⁰ NO diffuses into the dendrites of MSNs, which have soluble guanylyl cyclases (sGC) activated by NO production. In turn, activation of sGC stimulates the synthesis of the second messenger cGMP. In the intact striatum, transient changes of intracellular cGMP modulate neuronal excitability as well as short- and long-term glutamatergic corticostriatal transmission. Striatal DA depletion alters NO–sGC–cGMP signaling and contributes to the pathophysiological changes observed in basal ganglia circuits in PD. The NO–sGC–cGMP signaling pathways play an important role in basal ganglia dysfunction and the motor symptoms associated with PD and in L-dopa-induced dyskinesias, raising the possibility that this system might represent a possible target for neuroprotection.

FIG. 2. The importance of physiologic NMDA receptor subunit balance for motor activity and striatal synaptic plasticity. In physiologic condition (upper), a correct balance between NR2A and NR2B subunits of the NMDA receptor is associated with normal motor activity and with the ability of excitatory striatal synapses to induce long-term potentiation (LTP) after high-frequency stimulation (HFS) of corticostriatal fibers. In experimental models of early Parkinson's disease (middle), development of motor impairment is associated with an increase of the synaptic NR2A/NR2B subunit ratio and with altered expression of synaptic LTP after HFS. The treatment with TAT2A peptides (lower) targeting MAGUK–NR2A subunit interaction is able to ameliorate the clinical symptoms of the experimental disease, to normalize the synaptic NR2A/NR2B synaptic ratio, and to restore the synapses' ability to express LTP after HFS (MAGUK, membrane-associated guanylate kinases).

Behavioral studies have suggested a general facilitatory role of the striatal NO–sGC–cGMP system in locomotor activity⁵⁹; however, the scenario is more

complex in PD and in PD animal models.⁶⁰ The basal ganglia of PD patients^{61,62} as well as the striatum of DA-denervated animals show altered NO signaling.⁶³ Experimental data concerning striatal cGMP levels in PD models are not univocal, probably because of different measuring techniques and regions selected for analysis. Nevertheless, it is interesting to note that phosphodiesterase (PDE) mRNA, protein, and activity are elevated, suggesting that the metabolism of cGMP is increased in DA-depleted rats.^{63,64} There is a critical need for functional characterization of striatal activity of the various steps involved in this biochemical pathway such as NOS, sGC, cGMP, PKG, and distinct classes of PDEs. This characterization should be done in the same experimental models and at various times after DA denervation as well as following L-dopa treatment. In line with this idea, the role of the NO–sGC–cGMP system has recently been characterized in the induction of corticostriatal LTD in fully DA-denervated animals⁶⁵ (Fig. 3). The effect of cGMP-related signaling has also been recently investigated in transgenic animals expressing mutant α -synuclein, in particular in mice transgenic for mutant A53T- α -synuclein. These mice showed age-dependent biochemical alterations of the DA system and behavioral impairments.⁶⁶

NO leads to cell death and pathological plasticity through several pathways in addition to the activation of the c-GMP–PKG-related intracellular signal. NO participates in cell death signaling through cell stress and activation of inducible or neuronal nitric oxide synthase (NOS), leading to S-nitrosylation of

FIG. 3. Inhibition of phosphodiesterases rescues striatal long-term depression (LTD) in experimental models of Parkinson's disease (PD). Nitric oxide (NO) released by nitric oxide synthase (NOS)-positive interneurons activates soluble guanylyl cyclases (sGC), which stimulates the synthesis of the second-messenger cGMP, which, in turn, facilitates striatal LTD induction. Experimental models of PD are associated with the loss of LTD at glutamatergic striatal synapses onto spiny neurons. Treatment with inhibitors of phosphodiesterases (PDEs) is able to restore LTD induction at striatal synapses and to ameliorate motor performances (cGMP, cyclic GMP; DA, dopamine; DARPP32, dopamine and cAMP-regulated phosphoprotein 32 kDa; Glu, glutamate; MSN, medium spiny neuron; PDE, phosphodiesterases; PDE-inh, inhibitors of phosphodiesterases; PKG, protein kinase G).

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by NO. S-nitrosylation of GAPDH abolishes catalytic activity and confers on GAPDH the ability to bind to Siah1 (hereafter designated Siah), an E3-ubiquitin-ligase whose nuclear localization signal mediates nuclear translocation of the GAPDH/Siah complex. Interestingly, deprenyl and TCH346 at subnanomolar concentrations prevent S-nitrosylation of GAPDH, reduce GAPDH/Siah binding and block the nuclear translocation of GAPDH. These actions also occurred in an experimental model of PD at very low doses.⁶⁷

The Role of Parthanatos in Models of Parkinson's Disease

Poly(ADP-ribose) polymerase-1 (PARP-1), an important nuclear enzyme, is potently activated by DNA damage and facilitates DNA repair.^{68,69} Severe DNA damage, which is induced either by a variety of environmental stimuli such as the DNA-alkylating agent N-methyl-N'-nitro-N-nitrosoguanidin, free radicals, hydrogen peroxide, hydroxyl radical, peroxynitrite, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and ionizing radiation or by activation of glutamate receptors, leads to overactivation of PARP-1.^{69,70} Excessive activation of PARP-1 causes an intrinsic cell death program that is unique compared to apoptosis, necrosis, and autophagy and has been designated parthanatos.^{69,71} Parthanatos is widely involved in various diseases,⁷² including PD, stroke, trauma, ischemia reperfusion injury, Alzheimer's disease, multiple sclerosis, Huntington's disease, heart attack, and diabetes.

PAR polymer is produced by PARP-1 activation using nicotinamide adenine dinucleotide (NAD⁺) as the substrate and functions as a signal molecule to induce cell death.^{69,73-75} PAR polymer is potentially elevated in dopaminergic neurons and caused cell death in substantia nigra pars compacta after the treatment of mice with the neurotoxin MPTP.⁷⁶ PARP-1 knockout robustly protects dopaminergic neurons from MPTP neurotoxicity.⁷⁶ Moreover, pharmacological inhibition of PARP-1 also reduced α -synuclein- and 1-methyl-4-phenylpyridinium MPP⁺-induced cytotoxicity in PD *in vitro* models.⁷⁷ These studies indicate that parthanatos plays a pivotal role in dopaminergic neuron loss in different models of PD.

Parthanatos is mainly mediated by mitochondrial oxidoreductase apoptosis-inducing factor (AIF) release from the mitochondria and translocation to nucleus,⁷⁸ as AIF knockdown or neutralizing anti-AIF antibodies prevent AIF translocation to the nucleus and inhibit alkylating DNA damage-mediated cell death in a variety of experimental paradigms.^{69,73,79,80} Although the mechanisms of dopaminergic neuron loss in the MPTP animal models of PD are not yet fully understood, AIF has been implicated in mediating MPP⁺ toxicity in

dopaminergic cells, which was not inhibited by caspase inhibitors but was prevented by knockdown of AIF.⁸¹ Recent studies have advanced our understanding of the cross talk between mitochondria and the nucleus in parthanatos. On PARP-1 activation, PAR is formed and signals to mitochondria, where PAR interacts with AIF, disrupts AIF-mitochondria binding, and triggers AIF release from mitochondria and translocation to the nucleus, eventually causing cell death.⁷¹ However, an AIF mutant, which has no PAR-binding property, cannot be released from mitochondria and fails to translocate to the nucleus to mediate cell death after PARP-1 activation.⁷¹ In addition, a previously undescribed NMDA-induced cell survival molecule Iduna, encoded by *Rnf146*,⁸² was found to be a PAR-dependent ubiquitin E3 ligase. Iduna protects against NMDA excitotoxicity and stroke through preventing AIF nuclear translocation.^{82,83} Thus, Iduna functions as an endogenous inhibitor in parthanatos. Interestingly, it was found that PAR binding is required for Iduna's E3 ligase activity and neuroprotective effects. Therefore, targeting PAR signaling or PAR-AIF interaction could be a novel potential strategy to prevent dopaminergic neuron loss in PD.

LRRK2 Inhibition as a Potential Therapeutic for PD

Leucine-rich repeat kinase 2 (LRRK2) is a large multidomain protein comprising numerous functional domains including an ankyrin repeat region, a leucine-rich repeat domain, a Roc GTPase domain, a C-terminal of Ras domain, a kinase domain, and a WD40 domain.⁸⁴⁻⁸⁶ Studies have demonstrated that the LRRK2 protein is widely expressed in several organs as well as in the mammalian central nervous system, including the neurons of the striatum, cortex, substantia nigra, hippocampus, and cerebellum, where it associates with membranes and vesicular structures.⁸⁴⁻⁸⁷ Despite the identification of LRRK2's localization, distribution, and functional domains, the main physiological role of this protein and its physiological substrates remain largely unknown.^{85,88,89}

Currently, more than 40 LRRK2 mutations have been identified, most of which are a result of single amino acid substitutions.⁸⁹ The most prevalent LRRK2 mutation is an amino acid substitution whereby a highly conserved glycine residue (Gly²⁰¹⁹), in the kinase domain of LRRK2, is replaced by a serine residue.^{85,86,89,90} Several studies have demonstrated that this autosomal dominant mutation is associated with increased LRRK2 kinase activity, and it has also been linked to the majority of sporadic and familial cases of PD.^{84,86}

In an effort to compensate for the robust increase in kinase activity from the G2019S mutation in the

TABLE 1. Summary of confirmed LRRK2 kinase inhibitors

Compound name	Model system used	Reference
Staurosporin	In vitro kinase assay	86
		95
GF 109203X	In vitro kinase assay	86
Ro 31-8220	In vitro kinase assay	86
5-iodotubercidin	In vitro kinase assay	86
GW5074	In vitro kinase assay	86
	Primary cortical cultures	91
	In vivo	
Indirubin-3'-monooxime	In vitro kinase assay	86
	Primary cortical cultures	
	In vivo	
SP 600125	In vitro kinase assay	86
Damnacanthal	In vitro kinase assay	86
Raf inhibitor IV	In vitro kinase assay	86
ZM 336372	In vitro kinase assay;	86
	Primary cortical cultures	
Sorafenib	In vitro kinase assay;	86
	Primary cortical cultures	91
Raf-1 kinase inhibitor 1	In vitro kinase assay	95
G66976	In vitro kinase assay	95
K-252a	In vitro kinase assay	95
K-252b	In vitro kinase assay	95
H-1152	In vitro	89
		87
Sunitinib	In vitro	89
		87
LRRK2-IN-1	In vitro kinase assay	88
	In vitro	
	In vivo	
Y-27632	In vitro kinase assay	87
CZC-25146	In vitro kinase assay	90
CZC-54252	Primary neuronal cultures	
Hydroxyfasudil	In vitro kinase assay	87

Table represents a summary of LRRK2 kinase inhibitors which have been shown to robustly inhibit the catalytic activity of LRRK2 in a variety of model systems.

LRRK2 gene, numerous studies have focused on inhibiting LRRK2's increased kinase activity by means of small molecules.^{87–89,91,92} Myriad excellent studies have demonstrated that the use of pharmacological inhibitors such as the idoline compound GW5074 or the Raf kinase inhibitor sorafenib could not only inhibit LRRK2's autophosphorylation as well as its ability to phosphorylate several artificial substrates, but also significantly decrease neurotoxicity in both *in vitro* and *in vivo* model systems of PD^{88,93} (Table 1). Results such as those published by Lee and colleagues are highly promising, as they provide support that the inhibition of LRRK2 by small molecules may indeed present an avenue for the development of effective therapeutics for the treatment of PD.

However, caution has to be taken when considering the pharmacological inhibition of LRRK2 to treat PD. One main concern regarding the use of kinase inhibitors is the potential of off-target effects, as many kinase inhibitors not only inhibit LRRK2's kinase

activity but also inhibit multiple other kinases such as Rho kinases.⁸⁹ Although numerous published LRRK2 inhibitors successfully inhibited LRRK2's kinase activity *in vitro* and *in vivo*, the specificity and selectivity of several of these compounds for LRRK2 remains to be determined. In a recent study by Deng et al, the LRRK2 inhibitor LRRK2-IN-1 was found to selectively inhibit LRRK2 *in vitro* and *in vivo*.⁹⁰ Although the data presented by Deng et al are compelling, it remains unknown if the LRRK2 inhibitor LRRK2-IN-1 can also effectively and selectively inhibit the catalytic activity of LRRK2 in a mouse model or other *in vivo* model systems of PD. Moreover, the degree of LRRK2 inhibition required to have therapeutic potential has to be evaluated, as too little or too much inhibition of LRRK2 could elicit undesired side effects. In a recent study by Liu et al,⁹⁴ the authors demonstrated that LRRK2 deficiency results in the development of severe colitis in LRRK2-knockout mice.⁹⁴ In addition, in genetically engineered mice deficient in LRRK2, there were inclusions and potential injury in the proximal tubules of the kidney.^{95,96} These results further support the notion that the pharmacological interference with LRRK2's kinase activity as a means to treat PD is a very delicate fine-tuning act and that more research aimed at even better understanding the physiological role of LRRK2 is required.

Conclusions

Clinical trials failed in demonstrating clinical efficacy of drugs that in preclinical studies showed promising neuroprotective profiles in experimental models of PD. Nevertheless, neuroprotection in PD remains an important goal of current preclinical and clinical research. In fact, a successful neuroprotective treatment could dramatically ameliorate the quality of life of PD patients and improve the clinical efficacy of current symptomatic therapies. Present difficulties in achieving this goal include limited information on the mechanisms of neurodegeneration as well as problems in the methodology used to study disease progression. The failures of several potential neuroprotective therapies tested in the clinical studies could also be explained by the limitations of animal models on which the rationale to use a specific agent was tested. At present, animal models generated on the ground of both genetic and toxicological information of PD offer a wide repertoire of possibilities for the screening of putative neuroprotective drugs. Nevertheless, none of these models per se recapitulates the complex picture of the disease and its progression. Moreover, the identification of several genes implicated in PD has further supported the heterogeneity of this disorder, suggesting the idea that the neurodegeneration does not only involve the dopaminergic system but also affects other

anatomical sites. Among these sites, the striatum is a major candidate. In fact, the correct functioning of the dopaminergic system within the basal ganglia requires an intact target area represented by the striatum and its anatomical, synaptic, and molecular mechanisms. Thus, future neuroprotective strategies, in addition to the classical nigral “focus” should also consider the striatum as a novel target for neuroprotection.

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