[Frontiers in Bioscience, Elite, 7, 193-221, January 1, 2015]

Nitric oxide, a new player in l-dopa-induced dyskinesia?

Elaine Del Bel[1,2,3], Fernando E Padovan-Neto[2,3], Mariza Bortolanza[1,2], Vitor Tumas[2,3], Aderbal S Aguiar Junior[4], Rita Raisman-Vozari[5], Rui D Prediger[4]

[1]Department of Morphology, Physiology and Basic Pathology, School of Odontology of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil, [2]Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), Ribeirao Preto, SP, Brazil, [3]Department of Neuroscience and Behavior, University of Sao Paulo, Ribeirao Preto Medical School, Ribeirao Preto SP, Brazil, [4]Department of Farmacology, Center of Biological Sciences, Federal University of Santa Catarina, UFSC, Florianopolis, SC, Brazil, [5]INSERM UMR 1127, CNRS UMR 7225, UPMC, CRICM, Experimental therapeutics Neurodegeneration, Salpetriere Hospital – Batiment ICM (Institute of the Brain and Spinal Cord), Paris, France

TABLE OF CONTENTS

1. ABSTRACT

L-3,4-Dihydroxyphenylalanine (L-DOPA) remains the most effective symptomatic treatment of Parkinson's disease (PD). However, the long-term use of L-DOPA causes, in combination with disease progression, the development of motor complications termed L-DOPA-induced dyskinesia (LID). LID is the result of profound modifications in the functional organization of the basal ganglia circuitry. There is increasing evidence of the involvement of non-dopaminergic systems on the pathophysiology of LID. This raises the possibility of novel promising therapeutic approaches in the future, including agents that interfere with glutamatergic, serotonergic, adenosine, adrenergic, and cholinergic neurotransmission that are currently in preclinical testing or clinical development. Herein, we summarize the current knowledge of the pharmacology of LID in PD. More importantly, this review attempts to highlight the role of nitric oxide (NO) in PD and provide a comprehensive picture of recent preclinical findings from our group and others showing its potential involvement in dyskinesia.

2. INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive neurological disorder characterized by selective degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) and subsequent decrease of dopamine (DA) levels in the striatum. PD is now thought as a complex motor disorder related to degeneration of the DAergic system (featuring bradykinesia, tremor, rigidity and postural instability), but also a progressive multisystem disease with non-motor deficiencies (impaired olfaction, gastrointestinal,

genitourinary, cardiovascular and respiratory dysfunctions, sleep, sensory, visual and neuropsychiatric disorder). Although the motor symptoms of PD are well defined, the non-motor features of this disorder are under-recognized and, consequently, undertreated. Recent studies suggested a non-motor preclinical phase spanning up to 20 years or more (1, 2). The causes of non-motor symptoms in PD are multifactorial and partially linked to widespread distribution of alfa-synuclein (alfaSyn), the basic pathological protein aggregated in neurons, neurites, presynaptic terminals and glia as a hallmark in PD and other synucleinopathies (3). Non-motor symptoms may impair parkinsonian quality of life, particularly in advanced stages of the disease, and most of them do not respond to dopaminergic drugs. The pharmacotherapy of these motor and non-motor symptoms is complex and complicates long-term therapy of the disease, due to possible drug interactions and side effects. Moreover, antiparkinsonian compounds themselves contribute to the onset of some these motor and non-motor symptoms to a considerable extent (3, 4).

L-DOPA is the naturally occurring L-isomer of the amino acid D,L-dihydroxyphenylalanine. L-DOPA therapy has revolutionized the treatment of PD. Birkmayer and Hornykiewicz (5) carried out in 1961 the first clinical trial with L-DOPA that showed dramatic anti-akinetic effects after intravenous (i.v.) administration in PD patients. The use of L-DOPA in clinical routine became definitely established in 1967, when Cotzias and colleagues (6) introduced the chronic, high dose oral L-DOPA regimen, which is basically still practiced today. It provides marked motor symptomatic benefits to virtually all patients with PD. Although L-DOPA replacement therapy long-term use is associated with the appearance of motor complications including L-DOPA-induced dyskinesia (LID) and other side effects.

3. L-DOPA-INDUCED DYSKINESIA

There are many unsolved questions concerning the cause of L-DOPA treatment side effects. Several studies have examined the potential of L-DOPA to induce toxicity in normal animals and humans, as well as in animal models of PD. L-DOPA can generate cytotoxic reactive oxygen species (ROS) by way of the oxidative metabolism of DA or via autoxidation. L-DOPA can be toxic to cultured dopaminergic neurons (7). No reduction in the number of DAergic neurons was observed in the SNc of normal rats or mice chronically treated with high doses of L-DOPA (8). Similarly, there is no evidence of L-DOPA-induced neurodegeneration in normal primates (9) or non-parkinsonian humans (10). L-DOPA has also been tested in rats with dopaminergic lesions induced by 6-hydroxydopamine (6-OHDA) and instead of the previous toxicity observed in DA neurons in the ventral tegmental area (11) different studies showed that L-DOPA also promoted recovery of DA neurons with increased striatal innervation (12). Therefore, there is little or no evidence from *in vivo* studies to suggest that L-DOPA treatment damages nigral neurons in PD.

Indeed, there is evidence suggesting that under some circumstances L-DOPA might be protective and have trophic effects (13). Chronic L-DOPA administration promotes the expression of the trophic factor pleiotrophin in rats with moderate nigrostriatal lesions (14). Pleiotrophin, a secreted heparin-binding growth factor that is highly expressed during early post-natal brain development (15), has been shown to promote neurite outgrowth and the survival of DAergic neurons in embryonic mesencephalic cultures (16). Although the final conclusions of a recent clinical trial of L-DOPA in patients with PD remains unclear, the study did not show any clinical evidence that the drug has an adverse effect on disease progression (17). Further clinical and imaging studies to help clarify whether or not L-DOPA is toxic in PD are warranted. Therefore, L-DOPA remains as the ‘‘gold standard’’ for the treatment of patients with PD.

The occurrence of dyskinesia limits the ability to optimize the treatment regimen, affects functional disability, and impacts the patient quality of life. Hyperkinetic choreiform and/or dystonic abnormal involuntary movements characterize L-DOPA induced dyskinesia (LID). This might influence specific parts of the body or become generalized and seriously compromising. LIDS may affect up to 40% of PD patients treated with L-DOPA over a period of 5 years; the percentage of dyskinetic patients can reach up to 90% after a period of 10 years (18, 19). Table 1 summarizes clinical presentation and available treatments for LIDs in PD patients. Over the last years, new evidence has extended our understanding of the pathophysiology of LIDs and there is increasing evidence of the involvement of other non-dopaminergic systems (18). This raises the possibility for novel promising therapeutic approaches, including the

Table 1. Clinical presentations and current treatment strategies of L-DOPA-induced dyskinesia in patients with Parkinson's disease*

Type of dyskinesia	Temporal profile	Clinical aspects	Treatment strategies
Peak-dose dyskinesia	Dyskinesias are present at the peak of plasma levels of L-DOPA	Predominantly asymmetric generalized choreiform type movements, being more prominent in the most affected side; After taking L-DOPA the patient begins to improve, soon thereafter he develops dyskinesias that after some time diminish or disappear (pattern IDI: "Improvement-Dyskinesia-Improvement)	Reduce any antiparkinsonian medication (reduce dopaminergic stimulation); Use smaller single-doses of L-DOPA; Add dopamine agonists and reduce L-DOPA (change for a more continuous profile of dopaminergic stimulation); Add anti-dyskinetic drugs (amantadine, buspirone or clozapine); Surgical treatment: pallidal deep brain stimulation (DBS) or pallidotomy; Or consider the use of other strategies for providing continuous dopaminergic stimulation (subcutaneous infusions of apomorphine, intrajejunal infusion of L-DOPA)
Diphasic dyskinesia	Dyskinesias appear at the onset and at the end of L-DOPA antiparkinsonian action, and cease or are reduced at the peak plasma levels of L-DOPA	Dyskinesias predominate in the lower limbs; Have a stereotypical characteristic with repetitive alternating movements; Are predominantly dystonic (pattern DID: "Dyskinesia-Improvement-Dykinesia")	Add dopamine agonists; Increase L-DOPA single-dose; Use of other strategies for providing continuous dopaminergic stimulation (subcutaneous infusions of apomorphine, intrajejunal infusion of L-DOPA)
Square-wave or continuous dyskinesia	Dyskinesias are present during all the time of the L-DOPA cycle	In general predominantly choreic movements mixed or followed by dystonic movements	Treated case-by-case
Off-dyskinesia	Dyskinesias appear when plasma L-DOPA concentrations are low in the early morning before the first L-DOPA intake, or at the end of the L-DOPA cycle (off state)	Painful, distressing and disabling dystonic posture or cramp; Generally occurring in the lower limbs; Present in the ¨off state¨	Take the first dose of L-DOPA while in bed; Add a controlled release L-DOPA preparation at bedtime; Add a dopamine agonist to the antiparkinsonian drug regimen
*Data was collected mostly from reference 21. Certain of these agents are approved or undergoing study in the United States or other countries			

use of agents that interfere with glutamatergic, serotonergic, adenosine, adrenergic, and cholinergic neurotransmission, which could be used to prevent or decrease the severity of LIDs.

The pathophysiology of LIDs is complex and many factors are involved (for recent reviews, see (19, 20)). The current strategies for prevention and management and of LIDs include: delaying the use of L-DOPA and initiating therapy with DAergic agonists, discontinuation or decreasing individual doses of L-DOPA and adjusting the schedule time of drug intake, and the use of antidyskinetic agents (21) (Table 1). For patients presenting with severe motor complications these drug interventions are usually insufficient, and more aggressive and expensive strategies may be indicated (22). Stereotactic interventions in the basal ganglia with the use of ablative techniques or deep brain stimulation (DBS) implantation are effective to reduce LIDs, but they have restricted criteria to be indicated and technical problems were commonplace and intensive postoperative monitoring is necessary (23). They are also not free of adverse effects and include cognitive decline, speech difficulty, instability, gait disorders and depression (24). In conclusion, the clinical therapeutic strategies to prevent or reduce the intensity of LIDs are still limited.

Experimental models of PD have assisted in clarifying the anatomy and function of DAergic neurons as well as their relationship with the rest of the basal ganglia in the elaboration of motor responses (25, 26, 27). The neurotoxins 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and 6-OHDA are the two tools widely used to induce death of DAergic cells in the SNc although other animal models are currently used to simulate the neuropathological conditions of PD. These models have been also used to investigate new drug targets and therapeutic strategies (26, 28, 29). As observed in PD patients, administration of therapeutic doses of L-DOPA to parkinsonian rodents improves akinesia performance (30) but the chronicity of the treatment can induce the appearance of abnormal involuntary movements (AIMs) with similarities to LIDs observed in humans (31). The occurrence of LIDs is highly dependent on the degree of striatal denervation (32) and the dose of L-DOPA given (33).

The chronic administration of L-DOPA to 6-OHDA lesioned rodents causes the appearance of AIMs (31, 34, 35) that affect the axial, limb and orofacial muscles that can be rated on scales based on their topographical distribution, duration, and amplitude (32). In 1998, Cenci and collaborators (31) first published a rating scale to quantify L-DOPA-induced dyskinetic-like behaviors in rats. The validity of the model is exemplified by the fact that drugs used in the clinic for the treatment of LIDs can reduce the severity of AIMs in L-DOPA-treated animals (36). Pre- and post-synaptic molecular changes in striatum contribute to the establishment of LIDs (37). Among the molecular changes that occur in the striatum of rodents presenting AIMS are postsynaptic changes associated with DA-D1 receptors seem to be critical (38, 39) (see Figure 5). During PD progression, there is a gradual loss of DAergic neuronal loss and DA is no longer stored in presynaptic DAergic striatal terminals (40, 41).

The mechanisms behind LID are still not fully understood, but appear to involve maladaptive plasticity at striatal synapses. Down-regulation of DA autoreceptors (42) and DA release from non-DAergic terminals (43) will result in increased extracellular concentration of DA following L-DOPA administration (44). The association of all of these factors will result in a higher release of DA after L-DOPA administration leading to an increment of DA turnover (44, 45). Augmented DA turnover is thought to be one of the main precursors of LIDs (42). All these abnormal pre-synaptic-related alterations may induce altered stimulation of DA receptors and downstream post-synaptic changes. This idea is supported by the fact that continuous infusion of L-DOPA induces lower levels of dyskinesia (46). The increased density of DA receptors in striatum produces increments in second-messenger expression and changes in receptor trafficking (47, 48). Some downstream alterations includes the overexpression of the transcription factor FosB/DeltaFosB (35, 40, 50, 51, 52, 53, 54), phosphorylation of DARPP-32 at Thr-34 (55), phosphorylation of GluR1 subunit of AMPA receptor et serine 845 specific target of the protein kinase A (PKA; 60) and phosphorylation extracellular signal-regulated kinase (ERK) 1/2 (53) (see Figure 5).

More importantly, several pieces of evidence suggest that nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway is altered in PD (18). Herein, we summarize the current knowledge of the pharmacology of LIDs in PD. However, this article attempts to provide a comprehensive picture of the role of NO in PD and to highlight recent findings from our group and others showing its potential involvement in LIDs.

4. EMERGING TREATMENTS FOR L-DOPA-INDUCED DYSKINESIA: A BRIEFF SUMMARY

Once established, LIDs appear at every administration of L-DOPA and given that PD has still no cure, the search for new therapies that associated with L-DOPA prevent the onset of these motor complications are needed (56). The prevention and treatment of dyskinesia are a clinical unmet need and a great challenge for clinicians and basic scientists (57). Recently, some environmental changes such as physical exercise (53, 58) and pharmacological manipulation of non-dopaminergic neurotransmission systems have been showing great promise, such as glutamate, serotonin, adenosine and acetylcholine neurotransmission systems (19). Overall, these new agents seem to reduce the severity or extend the “on” time without LIDs (56). For most of the compounds promising results have been obtained in preclinical investigations and in initial clinical trials. However, long-term safety, tolerability and efficacy studies in patients are still required (19, 20,56, 57).

Multiple mechanisms underlying LIDs have been proposed but an efficient antidyskinetic therapy has not been developed thus far. Unfortunately, potential antidyskinetic effects observed in experimental models do not always translate into clinically useful effects (21, 58). Table 2 summarizes

Table 2. Drugs in clinical trials for the treatment of L-DOPA-induced dyskinesias in patients with Parkinson's disease*

Pharmacological class	Drug	Clinical findings	Reference
Glutamate NMDA antagonists	Amantadine	Effective against LIDs, controversy concerning the duration of antidyskinetic effect	(67, 68)
	Remacemide	No antidyskinetic effects	(19)
	Dextromethorphan	Reduced dyskinesia by 30-40%	(19)
	Memantine	Possibly effective against LIDs, good tolerability and safety	(44)
	Neu-120	Currently in phase II for safety, tolerability, pharmacokinetic and pharmacodynamic study	(45)
Glutamate AMPA antagonists	Perampanel	No antidyskinetic effects	(80,19)
	Talampanel	No findings available	(19, 20)
Glutamate mGluR antagonists	AFQ056	Reduced established LIDs, no negative effect on parkinsonian, safety and tolerability concerns	(76, 77, 19)
	Dipraglurant (ADX-48,621)	Improved parkinsonian and dyskinesia	(19)
alpha2-adrenergic receptor antagonists	Idazoxan	Controversial results concerning effectiveness and adverse-effects profile	(82, 83)
	Fipamezole	Partially effective against LIDs	(84)
Adenosine A2A receptor antagonists	Preladenant (Sch 420814)	Increase in dyskinesia rates, improvement in parkinsonian symptoms	(87)
	Istradefylline	Increase in dyskinesia rates, improvement in parkinsonian symptoms	(88)
	Tozadenant (SYN115)	No effect in dyskinesia, improvement in parkinsonian symptoms	(88)
Nicotine receptor agonists	Nicotine	Antidyskinetic, Serious adverse effects	(19, 20)
	SIB-1508Y	Very low tolerability	(19)
Monoamine oxidase-B inhibitors	Selegiline	Controversial results concerning effectiveness against LIDs	(62)
	Rasagiline	Partially effective against LIDs	(63)
	Safinamide	Improvement of LIDs	(66)
Partial dopamine receptor agonists	Aripiprazole	Effective against LIDs, well tolerated	(59)
	Pardoprunox	Effective against LIDs, improvement in parkinsonian symptoms	(60,61)
	Aplindore	No findings available	(19, 20)
Serotonin receptor agonists	Tandospirone	No effect in dyskinesia, worsening of parkinsonian symptoms	(19)
	Sarizotane	Controversial results concerning effectiveness against LIDs	(92, 19)
	Piclozotan	No findings available	(19, 20)
Other treatments	Zonisamide	Dose-dependent effectiveness against LIDs	(19)
	Gabapentin	No effect in dyskinesia	(19)
	Topiramate	No findings available	(19, 20)
	Valproate	No effect in dyskinesia	(20)
	Levetiracetam	Mild antidyskinetic effects	(20)

*LIDs: L-DOPA-induced dyskinesia; NMDA: N-methyl-d-aspartate; mGluR: AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; metabotropic glutamate receptors. For further information see (19, 20). The number between brackets correspond to the reference number

the main agents in clinical trials for the treatment of LIDs in PD.

4.1. Other Dopaminergic components

The use of partial DA receptors agonists has presented good results in the control of LIDs. Aripiprazole is a novel antipsychotic medication characterized by partial agonist of DAergic-D2 receptors and 5-HT1A receptors and by antagonism of 5-HT2A receptors (59). Aripiprazole, at very low doses, was well tolerated and reduced the severity of LIDs in PD patients (59). Pardoprunox (SLV308) is safe and improved clinical scores of PD patients, and improved the "on" time without troublesome LIDs (60, 61). The monoamine oxidase B (MAO-B) inhibition has shown intriguing antidyskinetic outcome despite raising central DA levels. For example, selegiline worse LIDs (62), while rasagiline increased daily "on" time without troublesome dyskinesia when used as an add-on therapy in patients with PD and motor fluctuations (63). Safinamide, a novel MAO-B and glutamate-release inhibitor, increased the duration of the antiparkinsonian response of L-DOPA (64). In PD patients, safinamide improved motor fluctuations (64) and "on" time without troublesome LIDs (65).

4.2. Glutamatergic component

The antidyskinetic effects of the low affinity N-Methyl-D-aspartate (NMDA) receptor antagonist amantadine aroused the interest of the role of glutamatergic neurotransmission in the development of LIDs. Amantadine is the best antidyskinetic drug indicated for the clinical management of LIDs (67, 68), and provides mild to moderate clinical benefits that usually decline with long-term therapy. The use of amantadine is limited due to its adverse effects (69). Amantadine is not universally effective can be poorly tolerated by some patients, and may elicit psychiatric complications. Although the exact antidyskinetic mechanism of amantadine is still not fully known, evidence has indicated a possible reduction of glutamatergic excitatory activity through the antagonism of NMDA receptors (70). Other glutamatergic antagonists advanced from experimental studies to clinical trials. Memantine showed no antidyskinetic effects in a double-blind crossover randomized study (71). Neu-120 is a potent selective and non-competitive NMDA receptor modulator currently in phase II for safety, tolerability, pharmacokinetic and pharmacodynamic study in patients with advanced PD and LIDs (72). Neu-120 showed antidyskinetic effect without prejudice to the antiparkinsonian effects of L-DOPA (72). The modulation of metabotropic glutamate receptor subtype 5 (mGlu5) has been investigated as a promising novel approach for the treatment of LIDs (73, 74, 75). Metabotropic mGlu5 receptor synergistically interacts with NMDA receptor to counteract dopaminergic dopamine D2 receptor signaling. Selective mGlu5 antagonists both prevent development and suppress expression of established dyskinesia (74). For example, the selective mGlu5 receptor antagonist AFQ056 has demonstrated antidyskinetic effects in experimental models of PD (74, 75). Two recently randomized controlled trials demonstrated antidyskinetic efficacy of AFQ056 in PD patients without changing the motor benefits of DAergic therapy (76, 77). However, PD patients treated with AFQ056 reported adverse effects such as dizziness, hallucination, fatigue, nasopharyngitis, diarrhea, and insomnia (76). Calcium-permeable AMPA receptors and splicing of AMPA receptors subunits were associated with LIDs phenotype in animal models (78). The selective AMPA receptor antagonist LY293558 reversed LIDs (79). The blockade of calcium entry with IEM 1460 showed similar antidyskinetic effects in animals (78, 79). Also there are few clinical trials testing AMPA receptor antagonists for the treatment of LIDs in patients with PD. Perampanel is a well-tolerated selective and noncompetitive AMPA receptor antagonist. But it was ineffective in improving motor symptoms of L-DOPA-treated patients with moderately advanced PD and motor fluctuations (80).

4.3. Adrenergic component

Many preclinical studies have demonstrated the role of alfa- and beta-adrenergic receptors in the development of LIDs. For instance, independent research groups have demonstrated antidyskinetic effects of adrenergic receptors antagonists such as idazoxan and fipamezole (JP-1730) in primates (81). Clinical results obtained so far are controversial. Manson *et al*. (82) showed the ineffectiveness of alfa2 receptor antagonist idazoxan against LIDs in PD patients. A single dose of idazoxan (20 mg) improved the severity of LIDs in a pilot randomized placebo-controlled study (83), without deterioration of the antiparkinsonian response to L-DOPA. Recently, a large double-blind randomized, placebo-controlled clinical trial provided evidence that fipamezole, a selective alfa2-adrenergic receptor antagonist, is well tolerated and reduced LIDs in PD patients (84). These results suggest that the use of alfa2-adrenergic receptor antagonists for the treatment of LIDs in these patients need to be further explored in animal models.

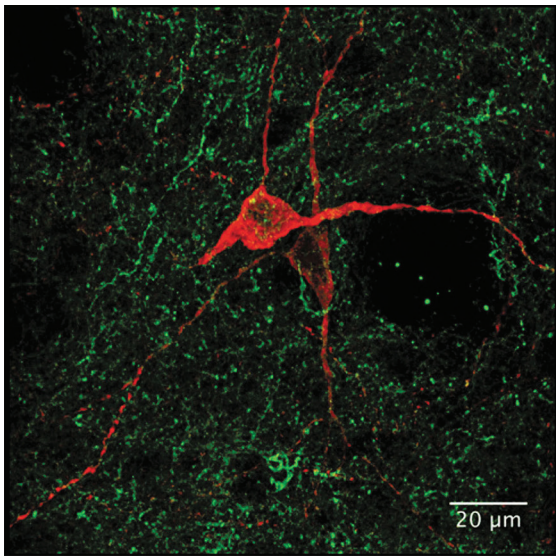

Figure 1. High resolution confocal laser scanning microscopy of TH- (green) and nNOS- (red) positive immunoreactivity in the rat striatum. The nNOS neurons terminals/fibers presented surrounded by a dense TH-ir positive neurons terminals. There is an absence of complete co-localization between nNOS and TH immunoreactivity in the rat striatum (for details see 102).

4.4. Adenosine component

Preclinical evaluation of A2A receptor antagonists was effective against LIDs (85,86). The well-known modulatory role of adenosine A2A receptors in the activity of DA-D2 receptors and mGlu5 receptors in the basal ganglia was also observed in experimental models of LIDs (79). However, clinical results with adenosine A2A receptor antagonists have not confirmed their promising potential for LIDs. A phase II, double-blind, randomized trial showed that the selective adenosine A2A receptor antagonist preladenant (SCH420814) worsened motor symptoms and dyskinesia of PD patients (87). Tozadenant (SYN115) and istradefylline (KW6002) relieved the motor fluctuations in PD patients, but they were ineffective against the troublesome LIDs (88). Istradefylline actually worsened “on” time with dyskinesia in patients (88).

4.5. Serotonergic component

Some authors have demonstrated the sprouting of striatal serotonergic nerve terminals in parkinsonian animals with LIDs (89, 90). The administration of buspirone, an agonist for the 5-HT1A autoreceptor, attenuates the development of LIDs (91). However, clinical studies have not obtained success in attenuating LIDs with the modulation of 5-HT1A autoreceptor; tandospirone a selective 5-HT1A receptor agonist did not show antidyskinetic effects in PD patients (120, 121). Sarizotan provided a significant reduction in troublesome dyskinesia when used as an adjunct to L-DOPA in PD patients (92). Eltoprazine, a drug that acts as an agonist on both 5-HT1A and 5-HT1B receptors and as an antagonist on 5-HT2C receptors, demonstrated excellent antidyskinetic effects, but decreased the antiparkinsonian effects of L-DOPA in animal models (93). In PD patients, low doses of clozapine (a partial agonist of 5-HT1A receptors), improved tremors (94), also presented a good antidyskinetic efficacy.

Among the various strategies investigated recently, the nitric oxide (NO)-cGMP system emerged as a new promising target for the treatment and understanding of LIDs (45, 54, 95, 96). In the next sessions we will review recent studies addressing these questions focusing on NO in addition to NO, DA and glutamate interactions in the striatum of parkinsonian rodents. Also, we will discuss the possible role of NO-cGMP system as a target for understanding the molecular basis of LIDs and the development of novel antidyskinetic agents (95, 96).

5. NITRIC OXIDE: SIGNALING PATHWAYS IN STRIATAL CIRCUITRY AND PARKINSON’S DISEASE

Nitric oxide (NO) is a small and highly diffusible molecule abundant in the striatal interneurons, besides different regions of the human body. Although first described as endothelium-derived relaxing factor in blood vessels (97), NO can act as a retrograde neurotransmitter or a signaling molecule in the central nervous system (CNS) (Figure 1), as well as antimicrobial and antitumor agent (98). Nowadays, NO has been associated with a variety of physiological and pathological process through his action on different cell types from neurons and glia to fibroblasts, myocytes and blood cells (99). As a neurotransmitter in the CNS, NO is known to work in an unorthodox way due to its biological characteristics: (i) it is synthesized postsynaptically in the cell bodies and dendrites, (ii) it is not stored in vesicles being a diffusible gas, (iii) it does not act at conventional receptors on the surface of adjacent neurons, (iv) it can act as a retrograde messenger diffusing to the presynaptic terminal.

Cytoplasmic nitric oxide synthase (NOS) is the enzyme responsible by NO production from the

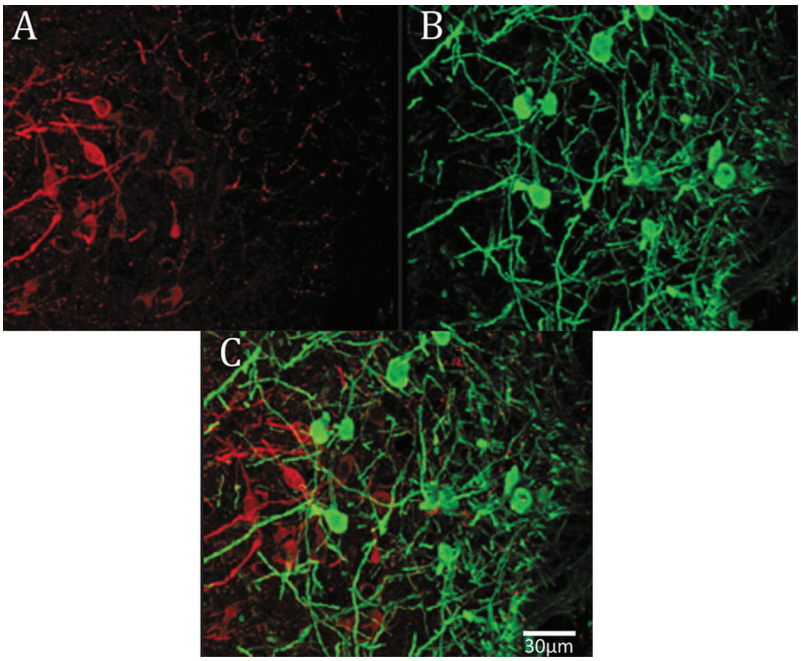

Figure 2. Distribution pattern of axons/cells immunoreactive for nNOS (red) and TH (green) in the rat substantia nigra in a double-stained section. The SNc is characterized by a high density of DAergic somata (green fluorescent cells-B and C), and a dense network of overlapping DAergic dendrites. Some nNOS-is neurons (red labeled) are also shown (A and C).

aminoacid L-arginine. Based on the first localization and signaling properties, three NOS isoforms can be recognized: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). The activity of eNOS and nNOS are both trigged in a calcium-dependent pathway, whilst in iNOS activity is calcium-independent. nNOS was first identified in CNS; it is also expressed in other tissues. It is abundant in different regions of the rat brain as showed in preparations using NADPH-diaphorase histochemistry techniques (100).

NOS-positive striatal interneurons have a relevant role in controlling motor activity (101). In basal ganglia, nNOS protein is present in several structures such as striatum (interneurons), substantia nigra (Figures 1, 2) and subthalamic nucleus. A proximity analysis of the dual localization of immunoreactivity for NOS and tyrosine hydroxylase (TH), enzymes responsible for the synthesis of NO and DA, respectively, was examined in regions of the nigroestriatal pathway (102). Co-localized regions were identified with a Pearson correlation coefficient ≥0.7. A large proportion of NOS positive immunoreactive soma/axon/dendrite themselves were directly opposed by TH-positive immunoreactive ones, within a radius of 1 and 2 µm (102- Figure 3). These anatomical arrangements corroborate evidence of DA and NO being intertwined in the anatomy in addition to physiology and pathology of the nigrostriatal pathway (56, 101, 103,104).

The striatum is the main structure of the basal ganglia where excitatory cortical information entry. According to the classic view of basal ganglia circuitry information arising from corticostriatal projections is processed within the striatum and transmitted to the output basal ganglia nuclei through medium spiny projection neurons. The excitatory cortical input to medium spiny neurons is modulated by many inputs, including NO produced by nNOS-expressing interneurons (105) and DA, arising from the DAergic terminals derived from the SNc. Due to the proximity of nitrergic, glutamatergic and DAergic nerve endings in striatum, a growing body of evidence has consistently shown that NO has an important modulatory role in the integration of information arising from the corticostriatal and nigrostriatal pathways (101) (see Figure 4).

nNOS-expressing striatal interneurons are gamma-aminobutyric acid (GABA) positive neurons also expressing neuropeptide Y (NPY) and somatostatin (105). These cells are aspiny with

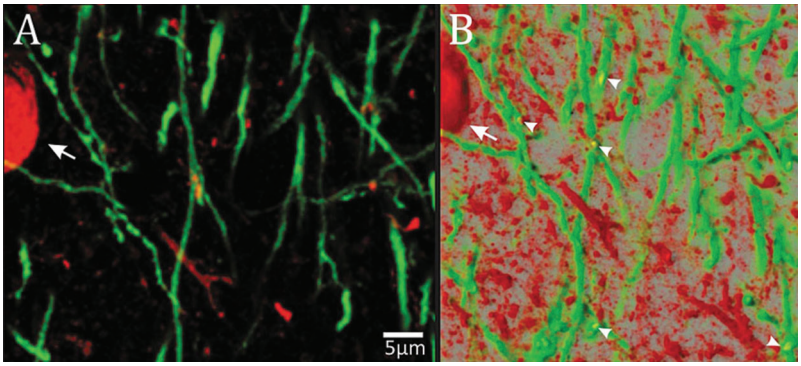

Figure 3. High resolution confocal laser scanning microscopy TH-positive fibers (green) and nNOS-positive (red) in a double-immunostained section from the rat substantia nigra (A). Image analysis might be best performed in three dimensions providing individual high resolution morphology. Using commercially available software Imaris (BitPlane) 3D reconstruction of the fluorescent image allowed us to reconstruct the filled neuron and process. We could determine the distribution of contacts between TH- and nNOS-cell body/process with les than 2um distance (B). The yellow dots (arrows head) indicate the location of TH-positive processes within the 2µm periphery of the nNOS-positive cell (for details see 102). Neuron body positive for nNOS is indicate by the arrow.

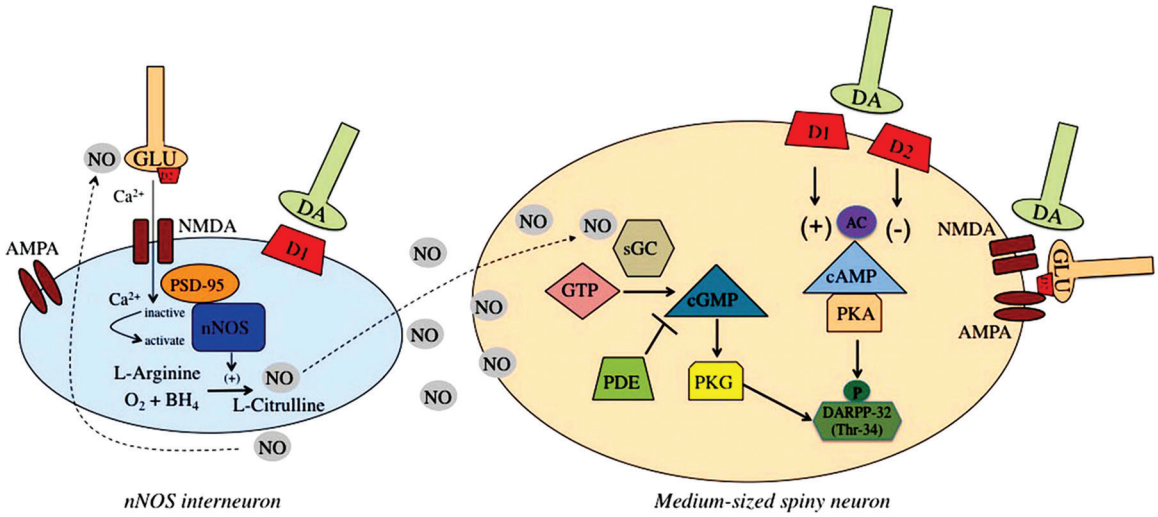

Figure 4. Schematic illustration of the effects of glutamate and dopamine on NO-signaling pathway in the striatum. Glutamate released from corticostriatal pathway facilitates calcium influx through NMDA receptors onto nNOS-expressing interneurons and promotes the activation of the nNOS enzyme. NO can act retrogradelly onto glutamatergic corticostriatal terminals or post-synaptically in the medium spiny neurons. The activation of soluble guanylate cyclase (sGC) within medium spiny neurons results in generation of cGMP, activation of cGMP-dependent protein kinases (PKG) and further effects in many downstream molecular signaling pathways, including the state of phosphorylation of DARPP-32. Dopamine seems to have a facilitatory action on NO-signaling pathway (and also downstream pathways) through D1/5 receptors, whereas activation of D2 dopaminergic pathway has the opposite effect. DA=dopamine, Ca^{2+}=calcium ion, D1=D1 dopamine receptor, D2=D2 dopamine receptor, GLU=glutamate, NMDA=N-methyl-D-aspartate receptor, AMPA=α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, O_2=molecular oxygen, BH_4=tetrahydrobiopterin.

12-25 mm in diameter, with fusiform or polygonal soma and represent 1-2% of striatal neurons. The nNOS enzyme is located on interneurons present in the dorsal striatum and the nucleus accumbens (100). The main intracellular target for NO is the soluble isoform of the enzyme guanylate cyclase (sGC). The striatum contains the highest concentration of this enzyme, which is located mainly in medium spiny neurons (106). The sGC acts as an effector molecule for NO, increasing the synthesis of the second messenger cGMP (107). cGMP activates cGMP-dependent protein kinases (PKG), phosphodiesterases (PDEs) and cGMP-regulated ion channels coupled to cGMP. PKG can

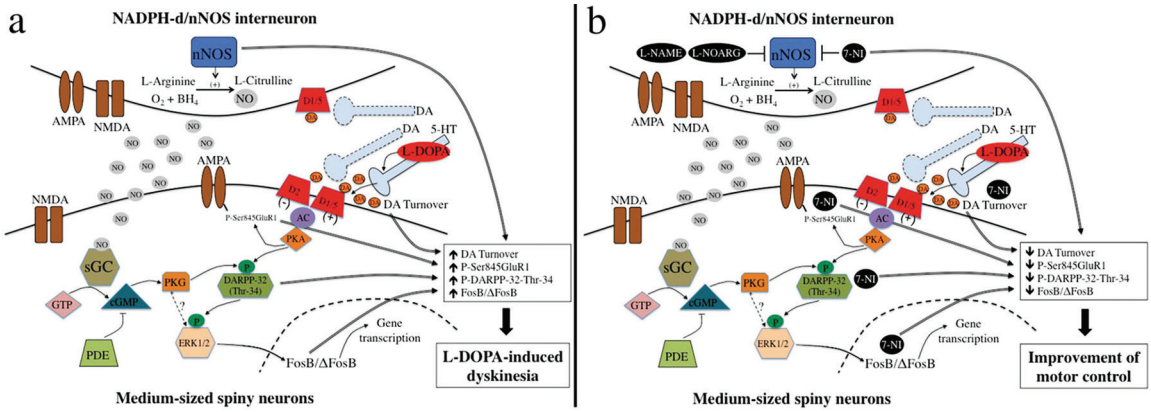

Figure 5. Impact of NOS inhibitors in NO-signaling and related pathways in L-DOPA-induced dyskinesia (LID) and possible role of NOS inhibitors as anti-dyskinetic compounds. (a) At the pre-synaptic sites, LID might be associated to the non-physiological release of DA from serotonergic terminals and increments the DA turnover. Several molecular markers of LID have been identified in medium spiny neurons. Some alterations include augmented phosphorylation events at GluR1 subunit of AMPA receptors at serine 845 and also at the amino acid threonine 34 (Thr-34) in DARPP-32 proteins. At the nuclei, there is an overexpression of the transcription factor FosB/DeltaFosB. The sum of these and other factors may be responsible for the appearance of unwanted abnormal involuntary movements. (b) Schematic representation of the anti-dyskinetic effects of NOS inhibitors in the molecular markers of LID, focusing on nNOS inhibition with 7-NI. The blockage of NOS enzyme with the nNOS inhibitor 7-NI reduces the augmented dopamine turnover, the abnormal phosphorylation of GluR1 subunit of AMPA receptors, the increased phosphorylation of DARPP-32 and FosB/DeltaFosB overexpression in the striatum. The precise mechanism of action of NOS inhibitors on these signaling pathways is not yet known. The role of these compounds on direct or indirect pathway is critical to the understanding the role of NO in LID. GTP=guanosine triphosphate, cGMP=cyclic guanosine monophosphate, PDE=phosphodiesterases, sGC=guanylate cyclase, PKG=cGMP-dependent protein kinase, PKA=protein kinase A, AC=adenylyl cyclase, 5-HT=serotonin, DA=dopamine, Ca^{2+}=calcium ion, D1=D1 dopamine receptor, D2=D2 dopamine receptor, GLU=glutamate, NMDA=N-methyl-D-aspartate receptor, AMPA=α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, O_2=molecular oxygen, BH_4=tetrahydrobiopterin, ERK=extracellular signal-regulated kinase

also phosphorylate dopamine- and cAMP-regulated phosphoprotein of 32-kDa (DARPP-32) in the amino acid threonine 34 (Thr-34) (110,109) and interact with many molecular signaling pathways. DARPP-32 is present in the cytoplasm and dendrites of medium spiny neurons (110) and is a potent inhibitor of protein phosphatase-1 (PP-1).

As illustrated in Figure 5, PP-1 enzyme regulates AMPA and NMDA receptors, the expression of transcription factors such as CREB (constitutive protein receptor binding to cAMP response element) and neural plasticity processes such as long-term depression (LTD) and long-term potentiation (LTP) (111). PDEs hydrolyze cyclic nucleotides (cAMP and cGMP), decreasing their availability. Isoforms of PDEs are present in medium spiny neurons; they consist of regulators of the signal transmitted by the NO-cGMP pathway (112).

Since NO is a gas, this molecule can influence the physiology of many others cells located at a relative distance (107). Under normal physiological conditions, activation of DA D1 or D2 receptors in striatum have opposite effects onto NO-cGMP pathway: the stimulation of D1 receptors increases the production of NO via nNOS (113) and also elevates the levels of cGMP (112), whereas activation of D2 receptors produces the opposed effect (113, 114) (Figure 4). In striatum, nNOS can be activated following a transient elevation in intracellular Ca^{2+} levels mediated through glutamate-induced activation of NMDA receptors (115). The interaction of nitrergic and glutamatergic system is complex and the role of NO-cGMP pathway in facilitating (116) or inhibiting (117) corticostriatal transmission is a debatable issue. Because NO interacts with DA (101) and glutamate in striatum (as well with other neurotransmitters), it is crucial to understand how the NO-cGMP pathway affects the transfer of information from the cortex to the basal ganglia through the striatum in neurodegenerative disorders such as PD.

Several pieces of evidence converging from different animal models of PD have suggested that alterations in NO-cGMP system may contribute to pathophysiological changes in basal ganglia following nigrostriatal DAergic pathway injury. Fully 6-OHDA-lesioned rats (i.e. presenting >95% of DAergic cell depletion in SNc) presented an increment in the total amount of nNOS protein in the striatum as evaluated

by western blot (95). Similarly, partial 6-OHDA-lesioned rats showed an increment in the number of NADPH-d/nNOS positive cells in the ipsilateral dorsal striatum (118, 119, 120). Similarly, MPTP administration to mice also induced up-regulation of nNOS-sGC-cGMP in the striatum (121). Moreover, the administration of rotenone (122) or manganese chloride (123) to rats significantly increased both the number of NADPH-d/nNOS-positive cells/fibers and the NOS activity in the striatum.

Taken together, the above observations strongly suggest that NO-cGMP pathway is altered in PD. Even though the exactly role of NO in PD remains unknown, some studies are now revealing that NO-cGMP pathway is an important modulator of plasticity in the parkinsonian striatum. Tseng *et al*. (124) demonstrated that the administration of the sGC inhibitor ODQ to 6-OHDA-lesioned rats attenuated the increments of cGMP levels in the striatum, the excessive abnormal firing of striatal medium spiny neurons and the increased metabolic activity of the subthalamic nucleus (STN). Furthermore, the authors also demonstrated that ODQ decreased akinesia in 6-OHDA-lesioned rats and in MPTP-treated mice. These results suggest that sGC-cGMP is related to dysfunctional basal ganglia signaling and the targeting of these molecules with new drugs may modify the disorganized activity of the striatal output pathways observed in PD. At cellular level, Yuste *et al*. (120) demonstrated that phosphorylation of DARPP-32 in Thr-34 (Thr-34-DARPP-32) was increased in the striatum of partially DA-depleted rats and correlated with the number of nNOS-positive interneurons. DARPP-32 plays an essential role in integrating signals from a number of behaviorally important neurotransmitters and neuromodulators that target the striatum (111). Interestingly, the administration of the preferential nNOS inhibitor 7-nitroindazole (7-NI) prevented the increase in the number of nNOS-expressing interneurons and the phosphorylation of DARPP-32 at Thr-34, suggesting that the state of phosphorylation of DARPP-32 in the parkinsonian striatum can be dependent of nNOS (120).

In agreement with these findings, several studies conducted in rodents without DAergic lesions indicate that pharmacological manipulations of NO-cGMP result in molecular and behavioral changes (103). For example, both systemic and intrastriatal administration of NOS and sGC inhibitors induce catalepsy in rodents (124, 125), an effect that is correlated with the reductions in striatal nitrite/nitrate levels (126).

Even thought, experimental data concerning striatal NO-cGMP levels in rodent models of PD are not univocal. Sancesario *et al*. (127) have described that the level of nNOS protein was decreased (42%) and the number of nNOS-immunopositive intrastriatal fibers (but not nNOS-immunopositive cell bodies) was markedly reduced in the striatum of 6-OHDA-lesioned rats. It was also demonstrated in rats that 6-OHDA-lesions of the nigrostriatal DAergic pathway resulted in a 50% decrease in the activity of this enzyme in the ipsilateral striatum and the frontal cortex (128). Furthermore, it was reported that nNOS-immunoreactive cells were unchanged in MPTP-treated mice (129). Discrepancies in results are common and may occur due to differences in utilized experimental models of PD, techniques and regions selected for analysis, but also to the degree of striatal DAergic lesion.

6. NITRIC OXIDE IN L-DOPA-INDUCED DYSKINESIA

There is a critical need for functional characterization of striatal activity of the NO-cGMP pathway in PD and the better understanding of the role of NO-cGMP pathway in PD may contribute for the development of new therapies aiming to restore motor function. Although scarce and sometimes contradictory, a growing body of evidence has shown that the NO system plays an important role in the pathophysiology of PD (35, 145, 146, 130). Studies conducted in parkisonian patients reported increments in the concentration of NO metabolites nitrate (131) and nitrite (132) and also nNOS overexpression in neutrophils (133). Furthermore, increased cGMP concentration in plasma (134, 135) and in cerebrospinal fluid (136) was found in PD patients treated with L-DOPA. Even thought, other reports suggested an opposed effect. Post-mortem studies in PD patients revealed a decrease of dendritic processes of NADPH-d positive striatal neurons (137), of striatal NADPH-d-containing cell numbers (138), and of nNOS mRNA expression (139. Also, it was reported a reduction in the concentration of cGMP in plasma and cerebrospinal fluid (140). The cellular signaling pathway of NO-cGMP in the striatum is complex, and several factors can interfere with the expression of the components of this pathway, especially in human studies. For example, levels of cGMP may change with age (141) and they are also altered in cardiovascular diseases (143).

Few studies were conducted so far in animal models of PD to analyze the effects of L-DOPA on

NO-GMPc system. Chalimoniuk and Langfort (142) demonstrated that MPTP-treated mice with partial lesion (40%) of DAergic neurons of the SNc presented up-regulation of nNOS-GC-beta1-cGMP levels in the striatum and midbrain but the amount of these molecules remained unchanged after chronic treatment with low and high L-DOPA doses (10 and 100 mg/kg, for 11-14 days). Even though, studies conducted in our laboratory demonstrated that chronic administration of L-DOPA increased both the number of nNOS-expressing interneurons and the total amount of nNOS protein as measured by western blot in dorsal striatum of fully 6-OHDA-lesioned rats, an effect that was additive to the lesion (unpublished results).

According to a nitrergic striatal dysfunction in LIDs, pioneering behavioral studies performed by our group demonstrated that NOS inhibitors are newly potential pharmacological approach for counteracting LIDs (54, 95, 96, 143). Combined therapies of L-DOPA and NOS inhibitors revealed interesting behavioral effects in rats and mice (101). Administration of an acute dyskinesiogenic dose of L-DOPA (100 mg/kg) to 6-OHDA-lesioned rats pre-treated with the non-selective NOS inhibitor NG-nitro-L-Arginine (L-NOARG) reduced abnormal involuntary movements, but impaired stepping test (95). Co-administration of the preferential nNOS inhibitor 7-NI and an acute dyskinesiogenic dose of L-DOPA (30 mg/kg) also reduced AIMs, with no observable motor impairment in rotarod performance and also in the stepping test (143).

In a different experimental paradigm, acute administration of NOS inhibitors after chronic L-DOPA treatment produced similar behavioral effects. Acute L-NOARG reduced pre-established LIDs in 6-OHDA-lesioned rats without interfering with stepping test performance (95, 96). The same effect was observed with 7-NI co-administration. 7-NI administration reduced pre-established LIDs in a dose-response manner and did not affect L-DOPA-induced motor improvement on the rotarod test (96).

Furthermore, chronic administration of 7-NI (during 8 days, starting at 26 days after initiation of chronic L-DOPA treatment) was able to reduce pre-established LIDs without interfering with rotarod and stepping test performance, indicating that chronic administration of 7-NI does not produce tolerance (143). Consistent with this data, we (unpublished observations) observed that chronic administration of the nonselective NOS inhibitor L-NAME and also 7-NI (administered 30 min before L-DOPA since the first day of treatment) were able to reduce LIDs in 6-OHDA-lesioned rats. The association of the iNOS inhibitor aminoguanidine did not interfere with LIDs, suggesting that the two other isoforms of the NOS enzyme are more linked to the appearance of LIDs.

nNOS is coupled to NMDA receptors thought the postsynaptic density protein (PSD-95) and activation of NMDA receptors is able to produce increments in striatal levels of NO thought nNOS (144, 145) (Figure 5). As mentioned before, overactivity of glutamatergic inputs in the basal ganglia has been suggested to be involved in the pathophysiology of LIDs. NMDA receptors antagonists such as amantadine, dextrorphan and dextromethorphan decrease LIDs in humans and in MPTP-treated primates (146, 147). Since activation of NMDA receptors augments neuronal calcium entry and stimulates nNOS, it is possible to speculate that one of the actions of NOS inhibitors is to reduce NMDA-induced increments of NO production. Even though, further studies addressing the specificity and targets of NOS inhibitors in LIDs are needed to understand their anti-dyskinetic effects.

Recently, some molecular changes related to NOS inhibitors are being revealed at both pre- and post-synaptic levels. Neurochemistry analysis performed by our group demonstrated that inhibition of pre-established AIMs with 7-NI (30 mg/kg) produces a markedly decrease in DA turnover in the striatum of dyskinetic rats (45). One possible explanation for the effects observed with 7-NI may be related to its ability to inhibit MAO-B. MAO-B is found attached to the membrane of the intraneuronal mitochondria and one of its functions is to convert DA in DOPAC (148). Thus, the antidyskinetic efficacy of 7-NI may also be associated with the inhibition of MAO-B (149, 150) since administration of 7-NI produces a significant increase in extracellular DA and a significant decrease in extracellular levels of DA metabolite dihydroxyphenylacetic acid (DOPAC) (151). Even thought, 7-NI-induced reduction of DA turnover may not only be related to MAO-B inhibition since it was reported that lower (10 and 25 mg/kg) but not high doses of 7-NI (50 mg/kg) failed to affect total MAO-A and MAO-B activity in striatum (152).

At post-synaptic sites, the anti-dyskinetic effects of NOS inhibitors impact on the expression of molecular markers of LIDs (see Figure 5). The transcription factor FosB/DeltaFosB is increased

in the DA-depleted striatum (35, 50, 53, 54), with similar patterns of expression observed in MPTP-treated monkeys (51) and PD patients (52). Following chronic L-DOPA treatment, FosB/DeltaFosB is selectively expressed in both dynorphinergic neurons (49, 50) and NADPH-d/NOS-positive striatal interneurons (35, 50). About 8% of rats (50) and 100% of mice (35) NADPH-d/NOS-positive interneurons in the striatum become FosB/DeltaFosB positive. Remarkably, the transgenic overexpression of this transcription factor in the striatum of 6-OHDA-lesioned rats chronically treated with L-DOPA was able to produce LIDs, indicating that FosB/DeltaFosB plays a crucial role in the expression of LIDs (153). While the mechanism underlying FosB/DeltaFosB expression in NOS-expressing interneurons during LIDs is unknown, it was observed that 7-NI-induced counteraction of pre-established LIDs (acute injection) decreased FosB/DeltaFosB overexpression in medial and lateral sectors of striatum (54). In agreement with that, the reduction of LIDs induced by chronic administration of 7-NI correlated with reduction of FosB/DeltaFosB overexpression in striatum as measured by western blot (154).

Increases in levels of DARPP-32 phosphorylation are reported in DA-depleted rodents (47, 55) exhibiting LIDs. DARPP-32 knockout mice display significantly less dyskinetic behavior in comparison to wild type counterparts (55). Overexpression of phosphorylated DARPP-32 may be due to sensitization of DA D1 receptors on medium spiny neurons of direct pathway induced by increased G protein coupling efficiency (47). NO is suggested to play a role in the abnormal levels of phosphor-DARPP-32-Thr34 in LIDs since chronic administration of 7-NI prior to L-DOPA inhibited L-DOPA-induced increases in phosphor-DARPP-32-Thr34 (110). In the striatum, PKG stimulates phosphorylation of DARPP-32 at Thr-34 (109). Thus, it is possible that the increased activity via NO-cGMP in dyskinetic animals produce increased phosphorylation of DARPP-32 at Thr-34.

Another possible postsynaptic target for NOS inhibitors could be the GluR1 AMPA receptor subunit. It was demonstrated that counteraction of LIDs by the NOS inhibitor 7-NI decreases the levels of phosphorylation of GluR1 subunit of AMPA receptors at Ser845 (110). The phosphorylation of GluR1 subunit of AMPA receptors at Ser845 is mediated by PKA, which also phosphorylates DARPP-32 at Thr34. PKA-mediated phosphorylation of GluR1 modulates glutamatergic transmission and is intensified by phospho-Thr34–DARPP-32 via inhibition of PP-1 (155, 156). Functionally, phosphorylation at Ser845 increases open AMPA channel probability (157) and surface expression (158) and may be related to long-term plasticity processes. Augmented AMPA receptor transmission may be involved in LIDs, as suggested by the ability of AMPA receptor agonists and antagonists to increase and decrease, respectively, LIDs in MPTP-treated monkeys (1591).

Striatal long-term plasticity such as LTP and LTD are altered in LIDs (87, 160) and can be influenced by the NO-cGMP pathway (117). LTD is absent following DA depletion in the striatum of rodent models of PD (161). It can be rescued by the administration of L-DOPA, but the induction of LTD is lost after the development of LIDs (160). The administration of PDEs inhibitors and exogenous cGMP were able to restore LTD in corticostriatal slices of dyskinetic rats but this effect that was blocked with intracellular inhibition of PKG (160), suggesting that the induction of LTD potentially occurs through cGMP-dependent mechanisms. In agreement with this hypothesis, biochemical analysis demonstrated that the peak of LIDs correlated with a bilateral decrease of cGMP in the cortico-striato-pallidal loop (162). Furthermore, subcutaneous (162) or intrastriatal (160) administration of the PDE inhibitor NO attenuated LIDs. Taken together, these studies reveal that synaptic plasticity associated with LIDs may be regulated by cGMP-dependent mechanisms.

On the other hand, data from inflammatory changes into postmortem brain of parkinsonian patients have been brought a new concept on the progression of PD. Microglial activation in PD patient brain was first reported in 1988 by McGeer and cols. (163) and then in 1991 by Forno and cols (164) in addition to Langston and coworkers descriptions from humans intoxicated with MPTP (165). Since then, a role of neuroinflammation in the progressive loss of DA neurons has been suggested (for review see (166)). Expression of iNOS has been detected in SNc from post-mortem PD patients (167) as well as in animal models of PD, (168, 169, 170). Once induced, iNOS produces sustained high levels of NO, which leads to neurotoxicity via the production of the free radical peroxynitrite causing DNA damage in PD (171).

In concordance with this neuroinflammatory mechanism of LIDs (172) amantadine, the only approved antidyskinetic drug for clinical use (173)

has been shown to induce anti-inflammatory effects (174). It is due to its capacity, at least in vitro studies, to reduce the release of pro-inflammatory factors from activated microglia and increase the expression of glial cell-derived neurotrophic factor (GNDF) from astrocytes (184). Then, as suggested by Del Bel *et al*. (103), the 7-NI and amantadine may have a similar anti-inflammatory mechanism on LIDs.

7. CLINICAL CONSIDERATIONS

Translational research is the process of transforming such discoveries into human application. There is compelling basic science supporting a role for non-dopaminergic approaches to LID but at the moment the translational benefit to PD is not being achieved as predicted. Clinical trial failures might result from a number of factors including inherent limitations of the models, over-interpretation of preclinical results and the complex nature of clinical trials for central nervous system disorders (for a recent review see 175). How in future, both experimental models of dyskinesia and clinical trial design could be optimized to ensure success?

Concerning NO system we would like to do several considerations.

The nNOS inhibitor 7-NI appears to more potently attenuate LIDs compared to L-NOARG, reducing up to 100% of LIDs in 6-OHDA-lesioned rats (96, 144). This difference may be due to the chemical structure of these compounds. L-NOARG is a guanidine-substituted analogue of L-arginine that preferentially inhibits eNOS/nNOS isoforms over iNOS (204).

The vascular effect of eNOS inhibition in the brain should not be neglected since angiogenic processes are affected in LIDs and may contribute to the establishment of motor complications (177, 178). The imidazole derivate 7-NI is more selective for nNOS when administered *in vivo*, having little or no effect on vasopressor activity in rat and mice (179) suggesting that the antidyskinetic effects induced by this compound are mainly related to the blockage of NO production via nNOS.

In addition, NO pathway plays a critical role in coordinating pre- and postsynaptic alterations underlying long-term synaptic plasticity and memory formation (180, 181). Inhibition of both nNOS and eNOS are necessary for the deterioration of memory processes. In neurodegenerative diseases this inconvenience should be taken into account when the effects of NOS inhibitors are evaluated.

8. CONCLUSION

Dyskinesia appears as a complex phenomenon, and several systems are involved in their pathophysiology. L-DOPA-induced AIMs in rodents resemble L-DOPA-induced motor abnormalities in PD patients, being involuntary and quite disabling. Although it is difficult to pinpoint a single factor leading to the development and expression of LIDs, at the moment, our understanding of dyskinesia revolves around DA and glutamate transmissions on the striatum. Due to the proximity of nitrergic, glutamatergic and DAergic nerve endings in striatum, a growing body of evidence has consistently shown that NO has an important modulatory role in the integration of information arising from the corticostriatal and nigrostriatal pathways. Moreover, there is converging evidence from studies conducted in PD patients and animal models of PD showing that the NO availability is increased in PD and that striatal neuroplasticity events associated with LIDs may be regulated by NO-cGMP-dependent mechanisms. In agreement with the hypothesis that the NO system plays an important role in the pathophysiology of LIDs, we reviewed pioneering behavioral studies performed by our group and others showing that NOS inhibitors are newly potential pharmacological approach for counteracting LIDs. Anti-dyskinetic effects of NOS inhibitors have been well characterized in preclinical investigations (rodent- 54, 95, 96, 101, 103,143,154; non-human primate-120). This processes in human remains to be proved.

The search for effective and selective NOS inhibitors is an important goal for pharmacotherapy. If we think that this could be transported to human there are several major issues that one should keep in mind when developing future clinical trials dealing with nitrergic system modulation. Localization of NOS neurons is widespread in human brain areas, including limbic and cortical areas that are implicated in memory and affective functions. Thus, the potential development of this NO modulation strategy against LIDs, especially for prolonged periods of treatment, would necessarily require a careful, detailed monitoring for cognitive and psychiatric problems in PD patients because most are already highly vulnerable to these nonmotor

alterations. Also, selective inhibition of nNOS or iNOS that not affect the endothelial isoform of NOS will be necessary in order to avoid the cardiovascular liabilities associated with endothelial NOS.

Future research will help to unravel the synaptic and molecular mechanisms underlying the modulation of NO system can protect against the development of LIDs. These findings may have clinical importance and inhibitors of the NO synthesis might correspond to a new therapeutic approach for controlling complications of drug treatment in PD.

9. ACKNOWLEDGEMENTS

The authors thank the financial support and grants provided by the Brazilian agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), CAPES- COFECUB (France/Brazil 681/2010), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) Programa Ciências sem Fronteiras (Pesquisador Visitante Especial); Cátedra França-Universidade de São Paulo. The authors have no financial or personal conflicts of interest related to this study. EDB and RDP are CNPq Fellows. The authors are in debt with Dr. Miso Mitkoviski and Dra. Marcia Graeff which performed the confocal laser work.

10. REFERENCES

1. K. R. Chaudhuri, D. Healy and A. H. V. Schapira: The non-motor symptoms of Parkinson's disease. Diagnosis and management. Lancet Neurol, 5, 235-45 (2006)
DOI: 10.1016/S1474-4422(06)70373-8
2. R. Savica, W. A. Rocca and J. E. Ahlskog: When does Parkinson disease start? Arch Neurol, 67, 798-801 (2010)
3. W. J. Schulz-Schaeffer: The synaptic pathology of alpha-synuclein aggregation in dementia with Lewy bodies, Parkinson's disease and Parkinson's disease dementia. Acta Neuropathol, 120, 131-43 (2010)
DOI: 10.1007/s00401-010-0711-0
4. R. D. Prediger, F. C. Matheus, M. L. Schwarzbold M. L., M. M. Lima and M. A. Vital: Anxiety in Parkinson's disease: a critical review of experimental and clinical studies. Neuropharmacology, 62, 115-24 (2012)
DOI: 10.1016/j.neuropharm.2011.08.039
5 W. Birkmayer and O. Hornykiewicz: Der L-Dioxyphenylalanin (DOPA) - Effekt bei der Parkinson-Akinese. Wien Klin Wschr, 73, 787-88 (1961)
6. C. C. Cotzias, M. H. Van Woert and I. M. Schiffer: Aromatic amino acids and modification of parkinsonism. N Engl J Med, 276, 374-79 (1967)
DOI: 10.1056/NEJM196702162760703
7. C. Mytilineou, S. K. Han and G. Cohen: Toxic and protective effects of l-DOPA on mesencephalic cell cultures. J Neurochem, 61, 1470-8 (1993)
DOI: 10.1111/j.1471-4159.1993.tb13642.x
8. T. L. Perry, V. W. Yong, M. Ito, J. G. Foulks, R. A. Wall, D. V. Godin and R. M. Clavier: Nigrostriatal dopaminergic neurons remain undamaged in rats given high doses of l-dopa and carbidopa chronically. J Neurochem, 43, 990-3 (1984)
DOI: 10.1111/j.1471-4159.1984.tb12834.x
9. L. Lyras, B. Y. Zeng G. McKenzie, R. K. Pearce, B. Halliwell and P. Jenner: Chronic high dose l-DOPA alone or in combination with the COMT inhibitor entacapone does not increase oxidative damage or impair the function of the nigro-striatal pathway in normal cynomolgus monkeys. J Neural Transm, 109, 53-6 (2002)
DOI: 10.1007/s702-002-8236-2
10. N. Quinn, D. Parkes, I. Janota and C. D. Marsden: Preservation of the substantia nigra and locus coeruleus in a patient receiving levodopa (2 kg) plus decarboxylase inhibitor over a four-year period. Mov Disord, 1, 65-8 (1986)
DOI: 10.1002/mds.870010109
11. S. B. Blunt, P. Jenner and C. D. Marsden: Suppressive effect of l-dopa on dopamine cells remaining in the ventral tegmental area of rats previously exposed to the neurotoxin 6-hydroxydopamine. Mov Disord, 8, 129-33 (1993)

DOI: 10.1002/mds.870080202

12. M. G. Murer, G. Dziewczapolski, L. B. Menalled, M. C. García, Y. Agid, O. Gershanik and R. Raisman-Vozari: Chronic levodopa is not toxic for remaining dopamine neurons, but instead promotes their recovery, in rats with moderate nigrostriatal lesions. Ann Neurol, 43, 561-75 (1998)
DOI: 10.1002/ana.410430504

13. M. A. Mena, V. Davila and D. Sulzer: Neurotrophic effects of l-dopa in postnatal midbrain dopamine neuron/cortical astrocyte cocultures. J Neurochem, 69, 1398-408 (1997)
DOI:10.1046/j.1471-4159.1997.69041398.x

14. J. E. Ferrario, A. E. Rojas-Mayorquín, M. Saldaña-Ortega, C. Salum, M. Z. Gomes, S. Hunot and R. Raisman-Vozari: Pleiotrophin receptor RPTP-zeta/beta expression is up-regulated by L-DOPA in striatal medium spiny neurons of parkinsonian rats. J Neurochem, 107, 443-52 (2008)
DOI: 10.1111/j.1471-4159.2008.05640.x

15. T. Kinnunen, E. Raulo, R. Nolo, M. Maccarana, U. Lindahl and H. Rauvala H: Neurite outgrowth in brain neurons induced by heparin-binding growth-associated molecule (HB-GAM) depends on the specific interaction of HB-GAM with heparan sulfate at the cell surface. J Biol Chem, 271, 2243-8 (1996)
DOI: 10.1074/jbc.271.4.2243

16. S. Mourlevat, T. Debeir, J. E. Ferrario, J. Delbe, D. Caruelle, O. Lejeune, C. Depienne, J. Courty, R. Raisman-Vozari and M. Ruberg: Pleiotrophin mediates the neurotrophic effect of cyclic AMP on dopaminergic neurons: analysis of suppression-subtracted cDNA libraries and confirmation in vitro. Exp Neurol, 194, 243-54 (2005)
DOI: 10.1016/j.expneurol.2005.02.015

17. S. Fahn: Parkinson's Study Group. Results of the ELLDOPA (Earlier vs. Later Levodopa) study. Mov Disord, 17, 13-14 (2002)

18. P. Huot, T. H. Johnston, J. B. Koprich, S. H. Fox and J. M. Brotschie: The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease. Pharmacol Rev, 65, 171-222 (2013)
DOI: 10.1124/pr.111.005678

19. J.E. Ahlskog and M. D. Muenter: Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord, 16, 448-58 (2001)
DOI: 10.1002/mds.1090

20. P. Bargiotas and S. Konitsiotis: Levodopa-induced dyskinesias in Parkinson's disease: emerging treatments. Neuropsychiatr Dis Treat, 9, 1605-17 (2013)

21. M. D. Gottwald and M. J. Aminoff: Therapies for dopaminergic-induced dyskinesias in Parkinson disease. Ann Neurol, 69, 919-27 (2011)
DOI: 10.1002/ana.22423

22. G. Deuschl, C. Schade-Brittinger, P. Krack, J. Volkmann, H. Schäfer, K. Bötzel, C. Daniels, A. Deutschländer, U. Dillmann, W. Eisner, D. Gruber, W. Hamel, J. Herzog, R. Hilker, S. Klebe, M. Kloss, J. Koy, M. Krause, A. Kupsch, D. Lorenz, S. Lorenzl, H. M. Mehdorn, J. R. Moringlane, W. Oertel, M. O. Pinsker, H. Reichmann, A. Reuss, G. H. Schneider, A. Schnitzler, U. Steude, V. Sturm, L. Timmermann, V. Tronnier, T. Trottenberg, L. Wojtecki, E. Wolf, W. Poewe and J. Voges: German Parkinson Study Group, Neurostimulation Section. N Engl J Med, 355, 896-908 (2006)
DOI: 10.1056/NEJMoa060281

23. W. M. Schüpbach, N. Chastan, M. L. Welter, J. L. Houeto, V. Mesnage, A. M. Bonnet, V. Czernecki, D. Maltête, A. Hartmann, L. Mallet, B. Pidoux, D. Dormont, S. Navarro, P. Cornu, A. Mallet and Y. Agid Y: Stimulation of the subthalamic nucleus in Parkinson's disease: a 5 year follow up. J Neurol Neurosurg Psychiatry, 76, 1640-4 (2005)
DOI: 10.1136/jnnp.2005.063206

24. F. M. Weaver, K. Follett, M. Stern, K. Hur, C. Harris, W. J. Jr Marks, J. Rothlind, O. Sagher, D. Reda, C. S. Moy, R. Pahwa, K. Burchiel, P. Hogarth, E. C. Lai, J. E. Duda, K. Holloway, A. Samii, S. Horn, J. Bronstein, G. Stoner, J. Heemskerk, G. D. Huang, CSP 468 Study Group: Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial. JAMA, 301, 63-73 (2009) DOI: 10.1001/jama.2008.929

25. R. D. Prediger, A. S. Jr Aguiar, A. E. Rojas-Mayorquin, C. P. Figueiredo, F. C. Matheus, L. Ginestet, C. Chevarin, E. D. Bel, R. Mongeau, M. Hamon, L. Lanfumey and R. Raisman-Vozari: Single intranasal administration of 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice models early preclinical phase of Parkinson's disease. Neurotox Res, 17, 114-29 (2010) DOI: 10.1007/s12640-009-9087-0

26. R. D. Prediger, A. S. Jr Aguiar, E. L. Moreira, F. C. Matheus, A. A. Castro, R. Walz, A. F. De Bem, A. Latini, C. I. Tasca, M. Farina and Raisman-Vozari R: The intranasal administration of 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine (MPTP): a new rodent model to test palliative and neuroprotective agents for Parkinson's disease. Curr Pharm Des, 17, 489-507 (2011) DOI: 10.2174/138161211795164095

27. F. C. Matheus, A. S. Jr Aguiar, A. A. Castro, J. G. Villarinho, J. Ferreira, C. P. Figueiredo, R. Walz, A. R. Santos, C. I. Tasca and R. D. Prediger: Neuroprotective effects of agmatine in mice infused with a single intranasal administration of 1-methyl-4phenyl-1,2,3,6-tetrahydropyridine (MPTP). Behav Brain Res, 235, 263-72 (2012) DOI: 10.1016/j.bbr.2012.08.017

28. G. E. Meredith, P. K. Sonsalla and M. F. Chesselet: Animal models of Parkinson's disease progression. Acta Neuropathol, 115, 385-98 (2008) DOI: 10.1007/s00401-008-0350-x

29. S. Duty and P. Jenner: Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol, 164, 1357-91 (2011) DOI: 10.1111/j.1476-5381.2011.01426.x

30. M. Olsson, G. Nikkhah, C. Bentlage and A. Björklund: Forelimb akinesia in the rat Parkinsonian model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J Neurosci, 15 (5 Pt 2), 3861-75 (1995)

31. M. A. Cenci, C. S. Lee and A. Björklund: L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci, 10, 2694-706 (1998) DOI: 10.1046/j.1460-9568.1998.00285.x

32. C. Winkler, D. Kirik, A. Björklund and M. A. Cenci: L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. Neurobiol Dis, 10, 165-86 (2002) DOI: 10.1006/nbdi.2002.0499

33. H. S. Lindgren, D. Rylander, K. E. Ohlin, M. Lundblad and M. A. Cenci: The "motor complication syndrome" in rats with 6-OHDA lesions treated chronically with L-DOPA: Relation to dose and route of administration. Behav Brain Res, 177, 150-9 (2007) DOI: 10.1016/j.bbr.2006.09.019

34. J. L. Taylor, C. Bishop and P. D. Walker: Dopamine D-1 and D-2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat. Pharmacol Biochem Behav, 81, 887-93 (2005) DOI: 10.1016/j.pbb.2005.06.013

35. N. Pavón, A. Martín, A. Mendialdua and R. Moratalla: ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. Biol Psychiatry, 59, 64-74 (2006)

DOI: 10.1016/j.biopsych.2005.05.044

36. A. Dekundy, M. Lundblad, W. D(anysz and M. A. Cenci: Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. Behav Brain Res, 179, 76-89 (2007) DOI: 10.1016/j.bbr.2007.01.013
37. M. A. Cenci and M. Lundblad: Post- versus presynaptic plasticity in L-DOPA-induced dyskinesia. J Neurochem, 99, 381-92 (2006) DOI: 10.1111/j.1471-4159.2006.04124.x
38. M. Feyder, A. Bonito-Oliva and G. Fisone: L-DOPA-Induced Dyskinesia and Abnormal Signaling in Striatal Medium Spiny Neurons: Focus on Dopamine D1 Receptor-Mediated Transmission. Front Behav Neurosci, 5, 71 (2011) DOI: 10.3389/fnbeh.2011.00071
39. M. G. Murer and R. Moratalla: Striatal Signaling in L-DOPA-Induced Dyskinesia: Common Mechanisms with Drug Abuse and Long Term Memory Involving D1 Dopamine Receptor Stimulation. Front Neuroanat, 5, 51 (2011) DOI: 10.3389/fnana.2011.00051
40. A. Ulusoy, G. Sahin and D. Kirik: Presynaptic dopaminergic compartment determines the susceptibility to L-DOPA-induced dyskinesia in rats. Proc Natl Acad Sci U S A, 107, 13159-64 (2010) DOI: 10.1073/pnas.1003432107
41. M. R. DeLong and T. Wichmann: Circuits and circuit disorders of the basal ganglia. Arch Neurol, 64, 20-4 (2007) DOI: 10.1001/archneur.64.1.20
42. R. de la Fuente-Fernández, V. Sossi, Z. Huang, S. Furtado, J. Q. Lu, D. B. Calne, T. J. Ruth and A. J. Stoessl: Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. Brain, 127(Pt 12), 2747-54 (2004) DOI: 10.1093/brain/awh290
43. M. Carta, H. S. Lindgren, M. Lundblad, R. Stancampiano, F. Fadda and M. A. Cenci: Role of striatal L-DOPA in the production of dyskinesia in 6-hydroxydopamine lesioned rats. J Neurochem, 96, 1718-27 (2006) DOI: 10.1111/j.1471-4159.2006.03696.x
44. E. D. Abercrombie, A. E. Bonatz and M. J. Zigmond: Effects of L-dopa on extracellular dopamine in striatum of normal and 6-hydroxydopamine-treated rats. Brain Res, 525, 36-44 (1990) DOI: 10.1016/0006-8993(90)91318-B
45. E. Del-Bel, F. E. Padovan-Neto, R. E. Szawka, C. A. da-Silva, R. Raisman-Vozari, J. Anselmo-Franci, A. C. Romano-Dutra and F. S. Guimaraes. Counteraction by nitric oxide synthase inhibitor of neurochemical alterations of dopaminergic system in 6-OHDA-lesioned rats under L-DOPA treatment. Neurotox Res, 25, 33-44 (2014) DOI: 10.1007/s12640-013-9406-3
46. L. A. Smith, M. J. Jackson, G. Al-Barghouthy, S. Rose, M. Kuoppamaki, W. Olanow and P. Jenner: Multiple small doses of levodopa plus entacapone produce continuous dopaminergic stimulation and reduce dyskinesia induction in MPTP-treated drug-naive primates. Mov Disord, 20, 306-14 (2005) DOI: 10.1002/mds.20317
47. I. Aubert, C. Guigoni, K. Håkansson, Q. Li, S. Dovero, N. Barthe, B. H. Bioulac, C. E. Gross, G. Fisone, B. Bloch and E. Bezard: Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. Ann Neurol, 57, 17-26 (2005) DOI: 10.1002/ana.20296
48. C. Guigoni, E. Doudnikoff, Q. Li, B. Bloch and E. Bezard: Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. Neurobiol Dis, 26, 452-63 (2007) DOI: 10.1016/j.nbd.2007.02.001
49. M. Andersson, A. Hilbertson and M. A. Cenci: Striatal fosB expression is causally linked with l-DOPA-induced

abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol Dis, 6, 461-74 (1999)
DOI: 10.1006/nbdi.1999.0259

50. M. Cenci, A. Tranberg, M. Andersson and A. Hilbertson: Changes in the regional and compartmental distribution of FosB- and JunB-like immunoreactivity induced in the dopamine-denervated rat striatum by acute or chronic L-dopa treatment. Neuroscience, 94, 515-27 (1999)
DOI: 10.1016/S0306-4522(99)00294-8

51. O. Berton, C. Guigoni, Q. Li, B. H. Bioulac, I. Aubert, C. E. Gross, R. J. Dileone, E. J. Nestler and E. Bezard: Striatal overexpression of DeltaJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. Biol Psychiatry, 66, 554-61 (2009)
DOI: 10.1016/j.biopsych.2009.04.005

52. P. K. Tekumalla, F. Calon, Z. Rahman, S. Birdi, A. H. Rajput, O. Hornykiewicz, T. Di Paolo, P. J. Bédard and E. J. Nestler: Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. Biol Psychiatry, 50, 813-6 (2001)
DOI: 10.1016/S0006-3223(01)01234-3

53. A. S. Jr Aguiar, E. L. Moreira, A. A. Hoeller, P. A. Oliveira, F. M. Cordova, V. Glaser, R. Walz, R. A. Cunha, R. B. Leal, A. Latini and R. D. Prediger: Exercise attenuates levodopa-induced dyskinesia in 6-hydroxydopamine-lesioned mice. Neuroscience, 243, 46-53 (2013)
DOI:10.1016/j.neuroscience.2013.03.039

54. F. E. Padovan-Neto, N. R. Ferreira, D. de Oliveira-Tavares, D. de Aguiar, C. A. da Silva, R. Raisman-Vozari and E. Del Bel: Anti-dyskinetic effect of the neuronal nitric oxide synthase inhibitor is linked to decrease of FosB/DeltaFosB expression. Neurosci Lett, 541, 126-31 (2013)
DOI: 10.1016/j.neulet.2013.02.015

55. E. Santini, E. Valjent, A. Usiello, M. Carta, A. Borgkvist, J. A. Girault, D. Hervé, P. Greengard and G. Fisone: Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia. J Neurosci, 27(26), 6995-7005 (2007)
DOI: 10.1523/JNEUROSCI.0852-07.2007

56. P. Jenner: Molecular mechanisms of L-DOPA-induced dyskinesia. Nat Rev Neurosci, 9, 665-77 (2008)
DOI: 10.1038/nrn2471

57. W. G. Meissner, M. Frasier, T. Gasser, C. G. Goetz, A. Lozano, P. Piccini, J. A. Obeso, O. Rascol, A. Schapira, V. Voon, D. M. Weiner, F. Tison and E. Bezard: Priorities in Parkinson's disease research. Nat Rev Drug Discov, 10, 377-93 (2011)
DOI: 10.1038/nrd3430

58. Klein C, Krainc D, Schlossmacher MG, Lang AE. Translational research in neurology and neuroscience 2011: movement disorders. Arch Neurol. 68(6): 709-16 (2011)
DOI: 10.1001/archneurol.2011.11

59. G. Meco, P. Stirpe, F. Edito, C. Purcaro, M. Valente, S. Bernardi and N. Vanacore: Aripiprazole in L-dopa-induced dyskinesias: a one-year open-label pilot study. J Neural Transm, 116(7), 881-4 (2009)
DOI: 10.1007/s00702-009-0231-z

60. R. A. Hauser, J. Bronzova, C. Sampaio, A. E. Lang, O. Rascol, A. Theeuwes, S. V. van de Witte and G. Pardoprunox Study: Safety and tolerability of pardoprunox, a new partial dopamine agonist, in a randomized, controlled study of patients with advanced Parkinson's disease. Eur Neurol, 62(1), 40-8 (2009)
DOI: 10.1159/000216839

61. O. Rascol, J. Bronzova, R. A. Hauser, A. E. Lang, C. Sampaio, A. Theeuwes and S. V. van de Witte: Pardoprunox as adjunct therapy to levodopa in patients with Parkinson's disease experiencing motor fluctuations: results of a double-blind, randomized, placebo-controlled,

trial. Parkinsonism Relat Disord, 18(4), 370-6 (2012)
DOI: 10.1016/j.parkreldis.2011.12.006

62. A. J. Lees: Comparison of therapeutic effects and mortality data of levodopa and levodopa combined with selegiline in patients with early, mild Parkinson's disease. Parkinson's Disease Research Group of the United Kingdom. BMJ, 311(7020), 1602-7 (1995)
DOI: 10.1136/bmj.311.7020.1602

63. O. Rascol, D. J. Brooks, E. Melamed, W. Oertel, W. Poewe, F. Stocchi, E. Tolosa and L. s. group: Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. Lancet, 365(9463), 947-54 (2005)
DOI: 10.1016/S0140-6736(05)71083-7

64. L. Gregoire, V. A. Jourdain, M. Townsend, A. Roach and T. Di Paolo: Safinamide reduces dyskinesias and prolongs L-DOPA antiparkinsonian effect in parkinsonian monkeys. Parkinsonism Relat Disord, 19(5), 508-14 (2013)
DOI: 10.1016/j.parkreldis.2013.01.009

65. F. Stocchi, R. Borgohain, M. Onofrj, A. H. Schapira, M. Bhatt, V. Lucini, R. Giuliani, R. Anand and I. Study: A randomized, double-blind, placebo-controlled trial of safinamide as add-on therapy in early Parkinson's disease patients. Mov Disord, 27(1), 106-12 (2012)
DOI: 10.1002/mds.23954

66. R. Borgohain, J. Szasz, P. Stanzione, C. Meshram, M. Bhatt, D. Chirilineau, F. Stocchi, V. Lucini, R. Giuliani, E. Forrest, P. Rice, R. Anand and I. Study: Randomized trial of safinamide add-on to levodopa in Parkinson's disease with motor fluctuations. Mov Disord, 29(2), 229-37 (2014)
DOI: 10.1002/mds.25751

67. E. Luginger, G. K. Wenning, S. Bösch and W. Poewe: Beneficial effects of amantadine on L-dopa-induced dyskinesias in Parkinson's disease. Mov Disord, 15, 873-8 (2000)
DOI: 10.1002/1531-8257(200009)15:5<873::AID-MDS1017>3.0.CO;2-I

68. R. Pahwa, S. A. Factor, K. E. Lyons, W. G. Ondo, G. Gronseth, H. Bronte-Stewart, M. Hallett, J. Miyasaki, J. Stevens and W. J. Weiner: Quality standards submcommittee of the American Academy of Neurology. Neurology, 66, 983-95 (2006)
DOI: 10.1212/01.wnl.0000215250.82576.87

69. W. H. Timberlake and M. A. Vance: Four-year treatment of patients with parkinsonism using amantadine alone or with levodopa. Ann Neurol, 3, 119-28 (1978)
DOI: 10.1002/ana.410030206

70. F. Blandini and M. T. Armentero: New pharmacological avenues for the treatment of L-DOPA-induced dyskinesias in Parkinson's disease: targeting glutamate and adenosine receptors. Expert Opin Investig Drugs, 21, 153-68 (2012)
DOI: 10.1517/13543784.2012.651457

71. M. Merello, M. I. Nouzeilles, A. Cammarota and R. Leiguarda: Effect of memantine (NMDA antagonist) on Parkinson's disease: a double-blind crossover randomized study. Clin Neuropharmacol, 22, 273-6 (1999)

72. Neurim Pharmaceuticals Ltd. Safety, Tolerability, PK and PD Study of Neu-120 in the Treatment of Levodopa-induced Dyskinesia. In: ClinicalTrials.gov (Internet). Bethesda (MD): National Library of Medicine (US). 2014/01/24. Available from: http://clinicaltrials.gov/ct2/show/study/NCT00607451. NLM Identifier: NCT00607451.

73. P. Klivenyi and L. Vecsei: Novel therapeutic strategies in Parkinson's disease. Eur J Clin Pharmacol, 66(2), 119-25 (2010)
DOI: 10.1007/s00228-009-0742-4

74. F. Mela, M. Marti, A. Dekundy, W. Danysz, M. Morari and M. A. Cenci: Antagonism of metabotropic glutamate receptor type

5 attenuates l-DOPA-induced dyskinesia and its molecular and neurochemical correlates in a rat model of Parkinson's disease. J Neurochem, 101, 483-97 (2007)
DOI: 10.1111/j.1471-4159.2007.04456.x

75. D. Rylander, H. Iderberg, Q. Li, A. Dekundy, J. Zhang, H. Li, R. Baishen, W. Danysz, E. Bezard and M. A. Cenci: A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys. Neurobiol Dis, 39, 352-61 (2010)
DOI: 10.1016/j.nbd.2010.05.001

76. F. Stocchi, O. Rascol, A. Destee, N. Hattori, R. A. Hauser, A. E. Lang, W. Poewe, M. Stacy, E. Tolosa, H. Gao, J. Nagel, M. Merschhemke, A. Graf, C. Kenney and C. Trenkwalder: AFQ056 in Parkinson patients with levodopa-induced dyskinesia: 13-week, randomized, dose-finding study. Mov Disord, 28, 1838-46 (2013)
DOI: 10.1002/mds.25561

77. D. Berg, J. Godau, C. Trenkwalder, K. Eggert, I. Csoti, A. Storch, H. Huber, M. Morelli-Canelo, M. Stamelou, V. Ries, M. Wolz, C. Schneider, T. Di Paolo, F. Gasparini, S. Hariry, M. Vandemeulebroecke, W. Abi-Saab, K. Cooke, D. Johns and B. Gomez-Mancilla: AFQ056 treatment of levodopa-induced dyskinesias: results of 2 randomized controlled trials. Mov Disord, 26, 1243-50 (2011)
DOI: 10.1002/mds.23616

78. C. Kobylecki, M. A. Cenci, A. R. Crossman and P. Ravenscroft: Calcium-permeable AMPA receptors are involved in the induction and expression of l-DOPA-induced dyskinesia in Parkinson's disease. J Neurochem, 114(2), 499-511 (2010)
DOI: 10.1111/j.1471-4159.2010.06776.x

79. C. Perier, C. Marin, M. Bonastre, E. Tolosa and E. C. Hirsch: AMPA receptor antagonist LY293558 reverses preproenkephalin mRNA overexpression in the striatum of 6-OHDA-lesioned-rats treated with L-dopa. Eur J Neurosci, 16, 2236-40 (2002)
DOI: 10.1046/j.1460-9568.2002.02275.x

80. K. Eggert, D. Squillacote, P. Barone, R. Dodel, R. Katzenschlager, M. Emre, A. J. Lees, O. Rascol, W. Poewe, E. Tolosa, C. Trenkwalder, M. Onofrj, F. Stocchi, G. Nappi, V. Kostic, J. Potic, E. Ruzicka, W. Oertel and D. German Competence Network on Parkinson's: Safety and efficacy of perampanel in advanced Parkinson's disease: a randomized, placebo-controlled study. Mov Disord, 25, 896-905 (2010)
DOI: 10.1002/mds.22974

81. J. M. Savola, M. Hill, M. Engstrom, H. Merivuori, S. Wurster, S. G. McGuire, S. H. Fox, A. R. Crossman and J. M. Brotchie: Fipamezole (JP-1730) is a potent alpha2 adrenergic receptor antagonist that reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Mov Disord, 18, 872-83 (2003)
DOI: 10.1002/mds.10464

82. A. J. Manson, E. Iakovidou and A. J. Lees: Idazoxan is ineffective for levodopa-induced dyskinesias in Parkinson's disease. Mov Disord, 15, 336-7 (2000)
DOI: 10.1002/1531-8257(200003)15:2<336::AID-MDS1023>3.0.CO;2-R

83. O. Rascol, I. Arnulf, H. Peyro-Saint Paul, C. Brefel-Courbon, M. Vidailhet, C. Thalamas, A. M. Bonnet, S. Descombes, B. Bejjani, N. Fabre, J. L. Montastruc and Y. Agid: Idazoxan, an alpha-2 antagonist, and L-DOPA-induced dyskinesias in patients with Parkinson's disease. Mov Disord, 16, 708-13 (2001)
DOI: 10.1002/mds.1143

84. P. A. Lewitt, R. A. Hauser, M. Lu, A. P. Nicholas, W. Weiner, N. Coppard, M. Leinonen and J. M. Savola: Randomized clinical trial of fipamezole for dyskinesia in Parkinson disease (FJORD study). Neurology, 79, 163-9 (2012)
DOI: 10.1212/WNL.0b013e31825f0451

85. R. Grondin, P. J. Bedard, A. Hadj Tahar, L. Gregoire, A. Mori and H. Kase: Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology, 52, 1673-7 (1999)
DOI: 10.1212/WNL.52.8.1673

86. N. Jones, C. Bleickardt, D. Mullins, E. Parker and R. Hodgson: A2A receptor antagonists do not induce dyskinesias in drug-naive or L-dopa sensitized rats. Brain Res Bull, 98, 163-9 (2013)
DOI: 10.1016/j.brainresbull.2013.07.001

87. R. A. Hauser, M. Cantillon, E. Pourcher, F. Micheli, V. Mok, M. Onofrj, S. Huyck and K. Wolski: Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. Lancet Neurol, 10, 221-9 (2011)
DOI: 10.1016/S1474-4422(11)70012-6

88. Y. Mizuno, K. Hasegawa, T. Kondo, S. Kuno, M. Yamamoto and G. Japanese Istradefylline Study: Clinical efficacy of istradefylline (KW-6002) in Parkinson's disease: a randomized, controlled study. Mov Disord, 25, 1437-43 (2010)
DOI: 10.1002/mds.23107

89. M. Carta, T. Carlsson, D. Kirik and A. Bjorklund: Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain, 130(Pt 7), 1819-33 (2007)
DOI: 10.1093/brain/awm082

90. T. Carlsson, M. Carta, C. Winkler, A. Bjorklund and D. Kirik: Serotonin neuron transplants exacerbate L-DOPA-induced dyskinesias in a rat model of Parkinson's disease. J Neurosci, 27(30), 8011-22 (2007)
DOI: 10.1523/JNEUROSCI.2079-07.2007

91. A. Munoz, Q. Li, F. Gardoni, E. Marcello, C. Qin, T. Carlsson, D. Kirik, M. Di Luca, A. Bjorklund, E. Bezard and M. Carta: Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of L-DOPA-induced dyskinesia. Brain, 131(Pt 12), 3380-94 (2008)
DOI: 10.1093/brain/awn235

92. C. W. Olanow, P. Damier, C. G. Goetz, T. Mueller, J. Nutt, O. Rascol, A. Serbanescu, F. Deckers and H. Russ: Multicenter, open-label, trial of sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). Clin Neuropharmacol, 27(2), 58-62 (2004)
DOI: 10.1097/00002826-200403000-00003

93. E. Bezard, E. Tronci, E. Y. Pioli, Q. Li, G. Porras, A. Bjorklund and M. Carta: Study of the antidyskinetic effect of eltoprazine in animal models of levodopa-induced dyskinesia. Mov Disord, 28(8), 1088-96 (2013)
DOI: 10.1002/mds.25366

94. Low-dose clozapine for the treatment of drug-induced psychosis in Parkinson's disease. The Parkinson Study Group. N Engl J Med, 340(10), 757-63 (1999)
DOI: 10.1056/NEJM199903113401003

95. F. E. Padovan-Neto, M. B. Echeverry, S. Chiavegatto and E. Del-Bel: Nitric Oxide Synthase Inhibitor Improves De Novo and Long-Term l-DOPA-Induced Dyskinesia in Hemiparkinsonian Rats. Front Syst Neurosci, 5, 40 (2011)
DOI: 10.3389/fnsys.2011.00040

96. F. E. Padovan-Neto, M. B. Echeverry, V. Tumas and E. A. Del-Bel: Nitric oxide synthase inhibition attenuates L-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. Neuroscience, 159, 927-35 (2009)
DOI: 10.1016/j.neuroscience.2009.01.034

97. R. F. Furchgott and J. V. Zawadzki: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature, 288, 373-6 (1980)
DOI: 10.1038/288373a0

98. C. Nathan: Nitric oxide as a secretory product of mammalian cells. FASEB J, 6, 3051-64 (1992)

99. G. H. Doherty: Nitric oxide in neurodegeneration: potential benefits of non-steroidal anti-inflammatories.

Neurosci Bull, 27, 366-82 (2011)
DOI: 10.1007/s12264-011-1530-6

100. S. Vincent and H. Kimura: Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience, 46, 755-84 (1992)
DOI: 10.1016/0306-4522(92)90184-4

101. E. A. Del Bel, F. S. Guimaras, M. Bermudez-Echeverry, M. Z. Gomes, A. Schiaveto-de-Souza, F. Padovan-Neto, V. Tumas, A. P. Barion-Cavalcanti, M. Lazzarini, L. P. Nucci-da-Silva and D. de Paula-Souza: Role of nitric oxide on motor behavior. Cell Mol Neurobiol, 25, 371-92 (2005)
DOI: 10.1007/s10571-005-3065-8

102. M. Mitkovski, F. E. Padovan-Neto, R. Raisman-Vozari, L. Ginestet, C. A. da-Silva and E. A. Del-Bel: Investigations into Potential Extrasynaptic Communication between the Dopaminergic and Nitrergic Systems. Front Physiol, 3, 372 (2012)
DOI: 10.3389/fphys.2012.00372

103. E. Del-Bel, F. E. Padovan-Neto, R. Raisman-Vozari and M. Lazzarini: Role of nitric oxide in motor control: implications for Parkinson's disease pathophysiology and treatment. Curr Pharm Des, 17, 471-88 (2011)
DOI: 10.2174/138161211795164176

104. M. Pierucci, S. Galati, M. Valentino, V. Di Matteo, A. Benigno, A. Pitruzzella, R. Muscat and G. Di Giovanni: Nitric oxide modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders. CNS Neurol Disord Drug Targets, 10, 777-91 (2011)
DOI: 10.2174/187152711798072329

105. Y. Kawaguchi, C. J. Wilson, S. J. Augood and P. C. Emson: Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci, 18, 527-35 (1995)
DOI: 10.1016/0166-2236(95)98374-8

106. J. D. Ding, A. Burette, P. I. Nedvetsky, H. H. Schmidt and R. J. Weinberg: Distribution of soluble guanylyl cyclase in the rat brain. J Comp Neurol, 472, 437-48 (2004)
DOI: 10.1002/cne.20054

107. J. Garthwaite and C. L. Boulton: Nitric oxide signaling in the central nervous system. Annu Rev Physiol, 57, 683-706 (1995)
DOI: 10.1146/annurev.ph.57.030195.003343

108. K. Tsou, G. L. Snyder and P. Greengard: Nitric oxide/cGMP pathway stimulates phosphorylation of DARPP-32, a dopamine- and cAMP-regulated phosphoprotein, in the substantia nigra. Proc Natl Acad Sci U S A, 90, 3462-5 (1993)
DOI: 10.1073/pnas.90.8.3462

109. A. Nishi, Y. Watanabe, H. Higashi, M. Tanaka, A. C. Nairn and P. Greengard: Glutamate regulation of DARPP-32 phosphorylation in neostriatal neurons involves activation of multiple signaling cascades. Proc Natl Acad Sci U S A, 102, 1199-204 (2005)
DOI: 10.1073/pnas.0409138102

110. C. C. Ouimet, K. C. Langley-Gullion and P. Greengard: Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. Brain Res, 808, 8-12 (1998)
DOI: 10.1016/S0006-8993(98)00724-0

111. P. Greengard, P. B. Allen and A. C. Nairn: Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron, 23, 435-47 (1999)
DOI: 10.1016/S0896-6273(00)80798-9

112. F. S. Menniti, W. S. Faraci and C. J. Schmidt: Phosphodiesterases in the CNS: targets for drug development. Nat Rev Drug Discov, 5, 660-70 (2006)
DOI: 10.1038/nrd2058

113. S. Sammut, K. E. Bray and A. R. West: Dopamine D2 receptor-dependent modulation of striatal NO synthase activity. Psychopharmacology, 191, 793-803 (2007)

DOI: 10.1007/s00213-006-0681-z

114. A. Di Stefano, P. Sozio, I. Cacciatore, A. Cocco, G. Giorgioni, B. Costa, M. Montali, A. Lucacchini, C. Martini, G. Spoto, F. Di Pietrantonio, E. Di Matteo and F. Pinnen: Preparation and pharmacological characterization of trans-2-amino-5(6)-fluoro-6(5)-hydroxy-1-phenyl-2,3-dihydro-1H-indenes as D2-like dopamine receptor agonists. J Med Chem, 48, 2646-54 (2005)
DOI: 10.1021/jm040889k

115. J. Garthwaite: Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci, 27, 2783-802 (2008)
DOI: 10.1111/j.1460-9568.2008.06285.x

116. S. Sammut, S. Threlfell and A. R. West: Nitric oxide-soluble guanylyl cyclase signaling regulates corticostriatal transmission and short-term synaptic plasticity of striatal projection neurons recorded in vivo. Neuropharmacology, 58, 624-31 (2010)
DOI: 10.1016/j.neuropharm.2009.11.011

117. P. Calabresi, B. Picconi, A. Tozzi and M. Di Filippo: Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci, 30, 211-9 (2007)
DOI: 10.1016/j.tins.2007.03.001

118. M. Z. Gomes, R. Raisman-Vozari and E. A. Del Bel: A nitric oxide synthase inhibitor decreases 6-hydroxydopamine effects on tyrosine hydroxylase and neuronal nitric oxide synthase in the rat nigrostriatal pathway. Brain Res, 1203, 160-9 (2008)
DOI: 10.1016/j.brainres.2008.01.088

119. M. Z. Gomes and E. A. Del Bel: Effects of electrolytic and 6-hydroxydopamine lesions of rat nigrostriatal pathway on nitric oxide synthase and nicotinamide adenine dinucleotide phosphate diaphorase. Brain Res Bull, 62, 107-15 (2003)
DOI: 10.1016/j.brainresbull.2003.08.010

120. J. E. Yuste, M. B. Echeverry, F. Ros-Bernal, A. Gomez, C. M. Ros, C. M. Campuzano, E. Fernandez-Villalba and M. T. Herrero: 7-Nitroindazole down-regulates dopamine/DARPP-32 signaling in neostriatal neurons in a rat model of Parkinson's disease. Neuropharmacology, 63, 1258-67 (2012)
DOI: 10.1016/j.neuropharm.2012.07.031

121. M. Chalimoniuk, N. Lukacova, J. Marsala and J. Langfort: Alterations of the expression and activity of midbrain nitric oxide synthase and soluble guanylyl cyclase in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice. Neuroscience, 141, 1033-46 (2006)
DOI:10.1016/j.neuroscience.2006.04.022

122. Y. He, S. Z. Imam, Z. Dong, J. Jankovic, S. F. Ali, S. H. Appel and W. Le: Role of nitric oxide in rotenone-induced nigro-striatal injury. J Neurochem, 86, 1338-45 (2003)
DOI: 10.1046/j.1471-4159.2003.01938.x

123. S. Ponzoni, F. S. Guimarães, E. A. Del Bel and N. Garcia-Cairasco: Behavioral effects of intra-nigral microinjections of manganese chloride: interaction with nitric oxide. Prog Neuropsychopharmacol Biol Psychiatry, 24, 307-25 (2000)
DOI: 10.1016/S0278-5846(99)00090-1

124. K. Y. Tseng, A. Caballero, A. Dec, D. K. Cass, N. Simak, E. Sunu, M. J. Park, S. R. Blume, S. Sammut, D. J. Park and A. R. West: Inhibition of striatal soluble guanylyl cyclase-cGMP signaling reverses basal ganglia dysfunction and akinesia in experimental parkinsonism. PLoS One, 6, e27187 (2011)
DOI: 10.1371/journal.pone.0027187

125. E. A. Del Bel, C. A. da Silva, F. S. Guimarães and M. Bermúdez-Echeverry: Catalepsy induced by intra-striatal administration of nitric oxide synthase inhibitors in rats. Eur J Pharmacol, 485, 175-81 (2004)
DOI: 10.1016/j.ejphar.2003.11.071

126. E. A. Del-Bel, F. S. Guimarães, S. R. Joca, M. B. Echeverry and F. R. Ferreira: Tolerance to the cataleptic effect that

follows repeated nitric oxide synthase inhibition may be related to functional enzymatic recovery. J Psychopharmacol, 24, 397-405 (2010)
DOI: 10.1177/0269881108097717

127. G. Sancesario, M. Giorgi, V. D'Angelo, A. Modica, A. Martorana, M. Morello, C. P. Bengtson and G. Bernardi: Down-regulation of nitrergic transmission in the rat striatum after chronic nigrostriatal deafferentation. Eur J Neurosci, 20, 989-1000 (2004)
DOI: 10.1111/j.1460-9568.2004.03566.x

128. J. de Vente, M. van Ittersum, J. van Abeelen, P. C. Emson, H. Axer and H. W. M. Steinbusch: NO-mediated cGMP synthesis in cholinergic neurons in the rat forebrain: effects of lesioning dopaminergic or serotonergic pathways on nNOS and cGMP synthesis. Eur J Neurosci, 12, 507-519 (2000)
DOI: 10.1046/j.1460-9568.2000.00927.x

129. Y. Muramatsu, R. Kurosaki, T. Mikami, M. Michimata, M. Matsubara, Y. Imai, H. Kato, Y. Itoyama and T. Araki: Therapeutic effect of neuronal nitric oxide synthase inhibitor (7-nitroindazole) against MPTP neurotoxicity in mice. Metab Brain Dis, 17, 169-82 (2002)
DOI: 10.1023/A:1020025805287

130. S. P. Gupta, S. Yadav, N. K. Singhal, M. N. Tiwari, S. K. Mishra and M. P. Singh: Does Restraining Nitric Oxide Biosynthesis Rescue from Toxins-Induced Parkinsonism and Sporadic Parkinson's Disease? Mol Neurobiol, 49, 262-75 (2014)
DOI: 10.1007/s12035-013-8517-4

131. J. A. Molina, F. J. Jiménez-Jiménez, J. A. Navarro, C. Vargas, P. Gómez, J. Benito-León, M. Ortí-Pareja, E. Cisneros and J. Arenas: Cerebrospinal fluid nitrate levels in patients with Parkinson's disease. Acta Neurol Scand, 93, 123-6 (1996)
DOI: 10.1111/j.1600-0404.1996.tb00186.x

132. G. A. Qureshi, S. Baig, I. Bednar, P. Sodersten, G. Forsberg and A. Siden: Increased cerebrospinal fluid concentration of nitrite in Parkinson's disease. Neuroreport, 6, 1642-4 (1995)
DOI: 10.1097/00001756-199508000-00013

133. E. M. Gatto, N. A. Riobó, M. C. Carreras, A. Cherñavsky, A. Rubio, M. L. Satz and J. J. Poderoso: Overexpression of neutrophil neuronal nitric oxide synthase in Parkinson's disease. Nitric Oxide, 4, 534-9 (2000)
DOI: 10.1006/niox.2000.0288

134. M. Chalimoniuk and A. Stepien: Influence of the therapy with pergolide mesylate plus L-dopa and with L-dopa alone on serum cGMP level in PD patients. Polish Journal of Pharmacology, 56, 647-50 (2004)

135. B. Brodacki, M. Chalimoniuk, J. Wesołowska, J. Staszewski, S. J. Chrapusta, A. Stępień and J. Langfort: cGMP level in idiopathic Parkinson's disease patients with and without cardiovascular disease - A pilot study. Parkinsonism Relat Disord, 17, 689-92 (2011)
DOI: 10.1016/j.parkreldis.2011.07.003

136. J. A. Navarro, F. J. Jiménez-Jiménez, J. A. Molina, J. Benito-León, E. Cisneros, T. Gasalla, M. Ortí-Pareja, A. Tallón-Barranco, F. de Bustos and J. Arenas: Cerebrospinal fluid cyclic guanosine 3'5' monophosphate levels in Parkinson's disease. J Neurol Sci, 155, 92-4 (1998)
DOI: 10.1016/S0022-510X(97)00267-0

137. J. Mufson and M. M. Brandabur: Sparing of NADPH-diaphorase striatal neurons in Parkinson's and Alzheimer's diseases. Neuroreport, 5, 705-8 (1994)
DOI:10.1097/00001756-199402000-00011

138. R. Böckelmann, G. Wolf, G. Ransmayr and P. Riederer: NADPH-diaphorase/nitric oxide synthase containing neurons in normal and Parkinson's disease putamen. J Neural Transm Park Dis Dement Sect, 7, 115-21 (1994)
DOI: 10.1007/BF02260966

139. D. Eve, A. Nisbet, A. Kingsbury, E.

Hewson, S. Daniel, A. Lees, C. Marsden and O. Foster: Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. Brain Res Mol Brain Res, 63, 62-71 (1998)
DOI: 10.1016/S0169-328X(98)00259-9

140. R. H. Belmaker, R. P. Ebstein, J. Biederman, R. Stern, M. Berman and H. M. van Praag: The effect of L-dopa and propranolol on human CSF cyclic nucleotides. Psychopharmacology, 58, 307-10 (1978)
DOI: 10.1007/BF00427396

141. R. Cui, H. Iso, K. Yamagishi, T. Ohira, T. Tanigawa, A. Kitamura, M. Kiyama, H. Imano, M. Konishi and T. Shimamoto: Relationship of urinary cGMP excretion with aging and menopausal status in a general population. J Atheroscler Thromb, 16, 457-62 (2009)
DOI: 10.5551/jat.No083

142. M. Chalimoniuk, J. Langfort, N. Lukacova and J. Marsala: Upregulation of guanylyl cyclase expression and activity in striatum of MPTP-induced parkinsonism in mice. Biochem Biophys Res Commun, 324, 118-26 (2004)
DOI: 10.1016/j.bbrc.2004.09.028

143. N. Novaretti, F. E. Padovan-Neto, V. Tumas, C. A. da-Silva and E. A. Del Bel: Lack of tolerance for the anti-dyskinetic effects of 7-nitroindazole, a neuronal nitric oxide synthase inhibitor, in rats. Braz J Med Biol Res, 43, 1047-53 (2010)
DOI:10.1590/S0100-879X2010007500111

144. J. Garthwaite, S. L. Charles and R. Chess-Williams: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature, 336(6197), 385-8 (1988)
DOI: 10.1038/336385a0

145. D. S. Bredt and S. H. Snyder: Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci U S A, 87(2), 682-5 (1990)
DOI: 10.1073/pnas.87.2.682

146. P. J. Blanchet, S. Konitsiotis, E. R. Whittemore, Z. L. Zhou, R. M. Woodward and T. N. Chase: Differing effects of N-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. J Pharmacol Exp Ther, 290, 1034-40 (1999)

147. A. Hadj Tahar, L. Gregoire, A. Darre, N. Belanger, L. Meltzer and P. J. Bedard: Effect of a selective glutamate antagonist on L-dopa-induced dyskinesias in drug-naive parkinsonian monkeys. Neurobiol Dis, 15, 171-6 (2004)
DOI: 10.1016/j.nbd.2003.10.007

148. M. B. Youdim, D. Edmondson and K. F. Tipton: The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci, 7, 295-309 (2006)
DOI: 10.1038/nrn1883

149. M. T. Silva, S. Rose, J. G. Hindmarsh and P. Jenner: Inhibition of neuronal nitric oxide synthase increases dopamine efflux from rat striatum. J Neural Transm, 110(4), 353-62 (2003)
DOI: 10.1007/s00702-002-0791-7

150. K. Castagnoli, S. Palmer, A. Anderson, T. Bueters and N. Castagnoli: The neuronal nitric oxide synthase inhibitor 7-nitroindazole also inhibits the monoamine oxidase-B-catalyzed oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Chem Res Toxicol, 10, 364-8 (1997)
DOI: 10.1021/tx970001d

151. C. Desvignes, L. Bert, L. Vinet, L. Denoroy, B. Renaud and L. Lambás-Señas: Evidence that the neuronal nitric oxide synthase inhibitor 7-nitroindazole inhibits monoamine oxidase in the rat: in vivo effects on extracellular striatal dopamine and 3,4-dihydroxyphenylacetic acid. Neurosci Lett, 264, 5-8 (1999)
DOI: 10.1016/S0304-3940(99)00139-1

152. B. Thomas, K. S. Saravanan and K. P. Mohanakumar: *In vitro* and *in vivo* evidences that antioxidant action contributes to the neuroprotective effects

of the neuronal nitric oxide synthase and monoamine oxidase-B inhibitor, 7-nitroindazole. Neurochem Int, 52, 990-1001 (2008)
DOI: 10.1016/j.neuint.2007.10.012

153. X. Cao, T. Yasuda, S. Uthayathas, R. L. Watts, M. M. Mouradian, H. Mochizuki and S. M. Papa: Striatal overexpression of DeltaFosB reproduces chronic levodopa-induced involuntary movements. J Neurosci, 30(21), 7335-43 (2010)
DOI: 10.1523/JNEUROSCI.0252-10.2010

154. K. Takuma, T. Tanaka, T. Takahashi, N. Hiramatsu, Y. Ota, Y. Ago and T. Matsuda: Neuronal nitric oxide synthase inhibition attenuates the development of L-DOPA-induced dyskinesia in hemi-Parkinsonian rats. Eur J Pharmacol, 683, 166-73 (2012)
DOI: 10.1016/j.ejphar.2012.03.008

155. G. L. Snyder, P. B. Allen, A. A. Fienberg, C. G. Valle, R. L. Huganir, A. C. Nairn and P. Greengard: Regulation of phosphorylation of the GluR1 AMPA receptor in the neostriatum by dopamine and psychostimulants in vivo. J Neurosci, 20(12), 4480-8 (2000)

156. K. Hakansson, S. Galdi, J. Hendrick, G. Snyder, P. Greengard and G. Fisone: Regulation of phosphorylation of the GluR1 AMPA receptor by dopamine D2 receptors. J Neurochem, 96, 482-8 (2006)
DOI: 10.1111/j.1471-4159.2005.03558.x

157. T. G. Banke, D. Bowie, H. Lee, R. L. Huganir, A. Schousboe, S. F. Traynelis: Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. J Neurosci. 20(1): 89-102 (2000).

158. S. Mangiavacchi and M. E. Wolf: D1 dopamine receptor stimulation increases the rate of AMPA receptor insertion onto the surface of cultured nucleus accumbens neurons through a pathway dependent on protein kinase A. J Neurochem, 88, 1261-71 (2004)
DOI: 10.1046/j.1471-4159.2003.02248.x

159. S. Konitsiotis, P. J. Blanchet, L. Verhagen, E. Lamers and T. N. Chase: AMPA receptor blockade improves levodopa-induced dyskinesia in MPTP monkeys. Neurology, 54, 1589-95 (2000)
DOI: 10.1212/WNL.54.8.1589

160. B. Picconi, V. Bagetta, V. Ghiglieri, V. Paillè, M. Di Filippo, V. Pendolino, A. Tozzi, C. Giampà, F. R. Fusco, C. Sgobio and P. Calabresi: Inhibition of phosphodiesterases rescues striatal long-term depression and reduces levodopa-induced dyskinesia. Brain, 134, 375-87 (2011)
DOI: 10.1093/brain/awq342

161. P. Calabresi, P. Gubellini, D. Centonze, G. Sancesario, M. Morello, M. Giorgi, A. Pisani and G. Bernardi: A critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. J Neurosci, 19(7), 2489-99 (1999)

162. M. Giorgi, V. D'Angelo, Z. Esposito, V. Nuccetelli, R. Sorge, A. Martorana, A. Stefani, G. Bernardi and G. Sancesario: Lowered cAMP and cGMP signalling in the brain during levodopa-induced dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. Eur J Neurosci, 28, 941-50 (2008)
DOI: 10.1111/j.1460-9568.2008.06387.x

163. P. L. McGeer, S. Itagaki, E. G.McGee: Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. Acta Neuropathol. 76(6): 550-7 1988
DOI: 10.1007/BF00689592

164. L. S. Forno, L. E. DeLanney, I. Irwin, D. Di Monte, J. W. Langston: 1992. Astrocytes and Parkinson's disease. Prog Brain Res. 94, 429-36. Astrocytes and Parkinson's disease. Prog Brain Res.;94:429-36. (1992)
DOI: 10.1016/S0079-6123(08)61770-7

165. J. W. Langston, L. S. Forno, J. Tetrud, A. G. Reeves, J. A. Kaplan and D. Karluk: Evidence of active nerve cell degeneration in the substantia nigra of humans years after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine exposure. Ann

Neurol, 46, 598-605 (1999)
DOI: 10.1002/1531-8249(199910)46:4<598::AID-ANA7>3.0.CO;2-F

166. E. C. Hirsch and S. Hunot: Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol, 8, 382-97 (2009)
DOI: 10.1016/S1474-4422(09)70062-6

167. S. Hunot, F. Boissière, B. Faucheux, B. Brugg, A. Mouatt-Prigent, Y. Agid and E. C. Hirsch: Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. Neuroscience, 72, 355-63 (1996)
DOI: 10.1016/0306-4522(95)00578-1

168. M. M. Iravani, K. Kashefi, P. Mander, S. Rose and P. Jenner: Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience, 110, 49-58 (2002)
DOI: 10.1016/S0306-4522(01)00562-0

169. G. T. Liberatore, V. Jackson-Lewis, S. Vukosavic, A. S. Mandir, M. Vila, W. G. McAuliffe, V. L. Dawson, T. M. Dawson and S. Przedborski S: Inducible nitric oxide synthase stimulates dopaminergic neurodegeneration in the MPTP model of Parkinson disease. Nat Med, 5, 1403-9 (1999)
DOI: 10.1038/70978

170. L. Broom, L. Marinova-Mutafchieva, M. Sadeghian, J. B. Davis, A. D. Medhurst and D. T. Dexter: Neuroprotection by the selective iNOS inhibitor GW274150 in a model of Parkinson disease. Free Radic Biol Med, 50, 633-40 (2011)
DOI: 10.1016/j.freeradbiomed.2010.12.026

171. S. Hague, T. Peuralinna, J. Eerola, O. Hellström, P. J. Tienari and A. B. Singleton: Confirmation of the protective effect of iNOS in an independent cohort of Parkinson disease. Neurology, 62, 635-6 (2004)
DOI: 10.1212/01.WNL.0000110191.38152.29

172. C. J. Barnum, K. L. Eskow, K. Dupre, P. Blandino-Jr, T. Deak, C. Bishop: Exogenous corticosterone reduces L-DOPA-induced dyskinesia in the hemi-parkinsonian rat: role for interleukin-1beta. Neuroscience. 156(1): 30-41 (2008).
DOI: 10.1016/j.neuroscience.2008.07.016

173. W. Danysz, C. G. Parsons, J. Kornhuber, W. J. Schmidt and G. Quack: Aminoadamantanes as NMDA receptor antagonists and antiparkinsonian agents--preclinical studies. Neurosci Biobehav Rev, 21, 455-68 (1997)
DOI: 10.1016/S0149-7634(96)00037-1

174. B. Ossola, N. Schendzielorz, S. H. Chen, G. S. Bird, R. K. Tuominen, P. T. Männistö and J. S. Hong: Amantadine protects dopamine neurons by a dual action: reducing activation of microglia and inducing expression of GDNF in astroglia. Neuropharmacology, 61, 574-82 (2011)
DOI: 10.1016/j.neuropharm.2011.04.030

175. P McGonigle and B. Ruggeri: Animal models of human disease: challenges in enabling translation. Biochem Pharmacol.87(1):162-71 (2014)
DOI: 10.1016/j.bcp.2013.08.006

176. P. K. Moore and R. L. Handy: Selective inhibitors of neuronal nitric oxide synthase--is no NOS really good NOS for the nervous system? Trends Pharmacol Sci, 18, 204-11 (1997)

177. E. Westin, H. S. Lindgren, J. Gardi, J. R. Nyengaard, P. Brundin, P. Mohapel and M. A. Cenci: Endothelial proliferation and increased blood-brain barrier permeability in the basal ganglia in a rat model of 3,4-dihydroxyphenyl-L-alanine-induced dyskinesia. J Neurosci, 26(37), 9448-61 (2006)
DOI: 10.1523/JNEUROSCI.0944-06.2006

178. K. E. Ohlin, V. Francardo, H. S. Lindgren, S. E. Sillivan, S. S. O'Sullivan, A. S. Luksik, F. M. Vassoler, A. J. Lees, C. Konradi and M. A. Cenci: Vascular endothelial growth factor is upregulated by L-dopa in the parkinsonian brain: implications for the development of dyskinesia. Brain, 134(Pt 8), 2339-57 (2011)

DOI: 10.1093/brain/awr165

179. P. K. Moore, R. C. Babbedge, P. Wallace, Z. A. Gaffen and S. L. Hart: 7-Nitro indazole, an inhibitor of nitric oxide synthase, exhibits anti-nociceptive activity in the mouse without increasing blood pressure. Br J Pharmacol, 108, 296-7 (1993)
DOI: 10.1111/j.1476-5381.1993.tb12798.x

180. C. Doyle, C. Holscher, M.J. Rowan and R. Anwyl, The selective neuronal NO synthase inhibitor 7-nitro-indazole blocks both long-term potentiation and depotentiation of field EPSPs in rat hippocampal area CA1 in vivo, J. Neurosci. 16: 418–424 (1996)

181 C. Holscher, L. McGlinchey, R. Anwyl and M.J. Rowan, 7-Nitro indazole, a selective neuronal nitric oxide synthase inhibitor in vivo, impairs spatial learning in the rat, Learn. Mem. 2: 267–278 (1996)
DOI: 10.1101/lm.2.6.267

Key Words: Nitric Oxide (NO), Parkinson's disease, L-DOPA-induced dyskinesias, LIDs, Review

Send correspondence to: Elaine A Del Bel, Departamento de Morfologia, Fisiologia e Patologia Basica, Faculdade de Odontologia de Ribeirao Preto, Universidade de Sao Paulo, SP 14049-904, Brazil, Tel: 55 16 3602 4047, Fax: 55 16 3633 0999, E-mail: eadelbel@forp.usp.br