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

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. L-DOPA-induced dyskinesias: a brief summary
4. Emerging treatments for L-DOPA-induced dyskinesia
   4.1. Other Dopaminergic components
   4.2. Glutamatergic component
   4.3. Adrenergic component
   4.4. Adenosine component
   4.5. Serotonergic component
5. Nitric Oxide: signaling pathways in striatal circuitry and Parkinson’s disease
6. Nitric Oxide in L-DOPA-induced dyskinesia
7. Clinical Considerations
8. Conclusion
9. Acknowledgements
10. References

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 alpha-synuclein (alphaSyn), 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
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.

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

<table>
<thead>
<tr>
<th>Type of dyskinesia</th>
<th>Temporal profile</th>
<th>Clinical aspects</th>
<th>Treatment strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak-dose dyskinesia</td>
<td>Dyskinesias are present at the peak of plasma levels of L-DOPA</td>
<td>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”)</td>
<td>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)</td>
</tr>
<tr>
<td>Diphasic dyskinesia</td>
<td>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</td>
<td>Dyskinesias predominate in the lower limbs; Have a stereotypical characteristic with repetitive alternating movements; Are predominantly dystonic (pattern DID: “Dyskinesia-Improvement-Dyskinesia”)</td>
<td>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)</td>
</tr>
<tr>
<td>Square-wave or continuous dyskinesia</td>
<td>Dyskinesias are present during all the time of the L-DOPA cycle</td>
<td>In general predominantly choreic movements mixed or followed by dystonic movements</td>
<td>Treated case-by-case</td>
</tr>
<tr>
<td>Off-dyskinesia</td>
<td>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)</td>
<td>Painful, distressing and disabling dystonic posture or cramp; Generally occurring in the lower limbs; Present in the “off state”</td>
<td>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</td>
</tr>
</tbody>
</table>

*Data was collected mostly from reference 21. Certain of these agents are approved or undergoing study in the United States or other countries
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 presentingAIMs 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 BRIEFE 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 antidysonkinetic therapy has not been developed thus far. Unfortunately, potential antidysonkinetic 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*

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

*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 DAAergic 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.
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).

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).

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
NO inhibition decreases L-DOPA-induced dyskinesia

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 nigrostriatal 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 corticostratial 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
NO inhibition decreases L-DOPA-induced dyskinesia

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 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 less than 2μm 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.
NO inhibition decreases L-DOPA-induced dyskinesia

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 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, Ca²⁺=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, O₂=molecular oxygen, BH₄=tetrahydrobiopterin, ERK=extracellular signal-regulated kinase.

![Diagram](image)

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 other 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 Ca²⁺ 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 intrastratial 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 though, 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 intrastrital 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 inhibition decreases L-DOPA-induced dyskinesia

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 though, 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...
NO inhibition decreases L-DOPA-induced dyskinesia

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 (159).

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 intrastratial (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 antidysonkinetic 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 isofrom 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.

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