An update on the role of nitric oxide in the neurodegenerative processes of parkinson's disease

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REVIEW ARTICLE

An Update on the Role of Nitric Oxide in the Neurodegenerative Processes of Parkinson's Disease

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Dedicated to Prof. Ferid Murad on his 80th birthday

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Abstract: ***Background:*** The pathogenesis of Parkinson's disease (PD) is not fully understood. Together with some important physiological functions in the Central Nervous System (CNS), nitric oxide (NO) can have both, neuroprotective or neurotoxic actions, depending on its redox state. An important body of evidence suggests the involvement of NO in many of the processes leading to neurodegeneration in several neurological disorders including PD. ***Objective:*** The main aim of this review is to update the data regarding the possible involvement of NO in the pathogenesis of PD. ***Methods:*** We performed a literature review on neuropathological, biochemical and genetic studies in PD patients and in several experimental models of parkinsonism and role of NO in these models. ***Results:*** Both studies in humans and in experimental models of parkinsonism give support to the contribution of NO in excitotoxicity, inflammation, oxidative stress, mitochondrial function impairment, DNA damage, and S-nitrosylation of diverse proteins. The interaction of these mechanisms leads finally to neuronal death. The fact that selective of specific inhibitors of NO synthase (NOS, the enzyme responsible of NO synthesis) should prevent neuronal death through their actions of these pathogenic mechanisms supports the role of NO on PD as well. ***Conclusion:*** NO participates in the pathogenesis of PD by multiple mechanisms described in this review.

Keywords: Nitric oxide, nitric oxide synthase, Parkinson's disease, neurodegeneration, oxidative stress, excitotoxicity, inflammation, S-nitrosylation.

INTRODUCTION

Despite an important body of research reported in the last decades, the pathogenesis of Parkinson's disease (PD), which is likely related with the interaction of multiple mechanisms that finally lead to neuronal death (mainly but not exclusively in the *substantia nigra pars compacta*), remains unsolved. One of the main hypotheses is the contribution of oxidative stress processes, but mitochondrial dysfunction, excitotoxicity, inflammation, deficiency in trophic factors and in calcium-binding proteins, neuromelanin, and other putative mechanisms could also play a role [1].

Nitric oxide (NO) plays an important role in several physiological processes in humans (maintenance of vasodilatory tone, inhibition of platelet aggregation, mediation of macrophage cytotoxicity, neurotransmission - acting as a neuronal messenger -, learning and memory, synaptic plasticity in the adult brain –long term potentiation and long term depression phenomena, gastrointestinal motility, and neuroendocrine regulation) [2]. In addition, NO can act, depending on the

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redox state, their concentrations, or both, as a neurotoxin or as neuroprotective factor [2, 3]. The neurotoxic effects of NO, that could be related with its role in oxidative stress, mitochondrial dysfunction, excitotoxity and neuroinflammation, seem to play an important role in many neurodegenerative disorders, such as PD, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD), and multiple sclerosis (MS), and in stroke [2-4]. In addition, NO could play an important role in the pathophysiology of migraine [5]. In this review, we will focused on the possible role of NO in the pathogenesis of PD.

NEUROCHEMICAL FEATURES OF NITRIC OXIDE

Redox Forms of Nitric Oxide

Redox forms of NO include the NO free radical ($NO^{\cdot}$), the nitrosonium cation (NO^{+}) and the nitrosyl anion (NO^{-}). $NO^{\cdot}$ can react with the following compounds [2],

1) Superoxide anion (O_2^{-}), leading to the synthesis of the highly toxic free radical peroxynitrite anion ($OONO^{-}$).
2) Transition metals of metalloproteins.
3) Iron-sulfur centers of several proteins, including enzymes (S-nitrosylation).
4) Tyrosine ($NO^{\cdot}$ can nitrate tyrosine in the presence of superoxide-dismutase and transition metals).

The nitrosonium cation NO^{+} is able to react with aromatic compounds, nitrated amines, thiols, and groups rich in electrons, and to blockade N-methyl-D-aspartate (NMDA) glutamatergic receptors. Finally, NO^{-} oxidates thiol groups and forms complexes with [Fe(III)-haem] [2].

Synthesis of Nitric Oxide

The responsible enzymes of the synthesis of NO are the NO synthases (NOS). These enzymes catalyze the conversion of L-arginine to L-citrulline (producing NO) in the presence of molecular oxygen, nicotinamide-adenine-dinucleotide-phosphate (NADPH), tetrahydrobiopterin, and flavines (flavine-adenine-dinucleotide –FAD- and flavine-adenine mononucleotide - FMN) [2-4]. This reaction of synthesis or NO, which implies the transference of five electrons, is depicted in Fig. (**1**).

Three isoforms of NOS have been identified in the Central Nervous System, being two of them constitutive (NOS1, neuronal NOS or nNOS; and NOS3, endothelial NOS, or eNOS) and the other one inducible (NOS2, immunological NOS or iNOS). NOS1, NOS2, and NOS3 enzymes are encoded, respectively, by the genes *NOS1* or *nNOS*, *NOS2* or *iNOS*, and *NOS3* or *eNOS* [2-4, 6]. The main features of NOS isoforms and their respective coding genes are summarized in Table **1**.

It has been proposed (despite this issue is controversial) the existence of a mitochondrial isoform of NOS (mtNOS) because mitochondria are able to produce NO through a Ca^{2+} sensitive NO synthase. It has been re-

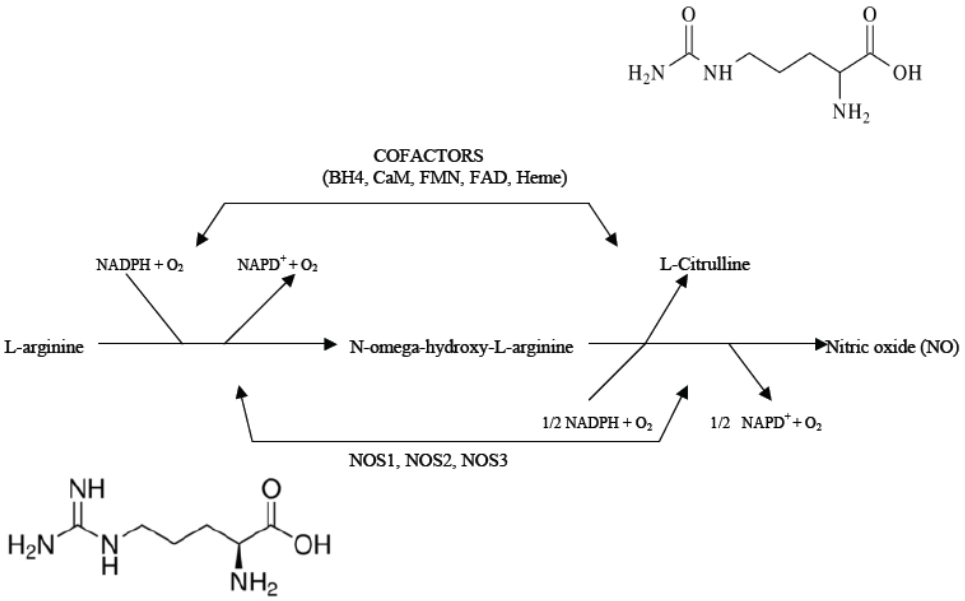

Fig. (1). Synthesis of nitric oxide.

Table 1. Isoforms of NO synthase (NOS) (information taken from references [2] and [3] and links http://www.ncbi.nlm.nih.gov/gene/4842, http://www.ncbi.nlm.nih.gov/gene/4843, and http://www.ncbi.nlm.nih.gov/gene/4846

NOS ISOFORM	GENE	CHROMOSOME/GENE IDENTITY/ MIM	MAIN LOCATION	MAIN FUNCTION	CALCIUM DEPENDENCE	EXPRESION IN THE CNS
Neuronal NOS (nNOS, NOS1)	*NOS1*	12q14.22/ 4842/ 163731	• Central Nervous System • Skeletal muscle (type II fibers)	• Cell communication	Yes	Neurons and, to a lesser extent, glial cells
Inducible NOS (iNOS, NOS2)	*NOS2*	17q11.2/ 4843/ 163730	• Immune system • Cardiovascular system	• Immunological defense against pathogen agents • It is nduced by proinflammatory cytokines and gram-negative endotoxins	No	Neurons, astrocytes, microglia, and endothelial cells (only under pathological conditions)
Entothelial NOS (eNOS, NOS3, cNOS)	*NOS3*	7q36/ 4846/ 163729	• Endothelium	• Vasodilatation	Yes	Endothelial cells and, to a lesser extent, some neuronal populations

ported a cross-reaction of mitochondria with antibodies to Ca^{2+}-sensitive eNOS, a similarity between mtNOS and nNOS in several tissues, and the absence of mtNOS activity in mitochondria isolated from heart of mice knockout for *nNOS* but not in mice knockout for *eNOS* and *iNOS*, suggesting the relation of mtNOS with nNOS [7].

Nitric Oxide in the Central Nervous System (CNS)

The findings that NMDA receptor agonists caused the release of a substance with certain similarities to the so called "endothelium derived relaxing factor" (EDRF), and the demonstration of the presence of NOS activity in the rat brain, both reported at the end of the 1980s, were the first evidences of NO synthesis in the CNS. Besides the activation of NMDA receptors, NO synthesis can be elicited by non-NMDA glutamate receptors, acetylcholine, angiotensin, bradykinin, serotonin, neurotensin and endotheline. NO is produced not only by neurons, but also by migroglia and astrocytes (revised in reference [2]). Table **2** summarizes localizations of NOS in the CNS.

Mechanisms of NO toxicity

The main physiological action of NO is the activation of the soluble guanylyl cyclase (sGC), which leads to the synthesis of guanosine 3'5'-cyclic monophosphate (cGMP). The enzyme sGC contains two subunits, designed as alpha and beta, and a prosthetic heme group with a ferrous iron. The actions of cGMP include the regulation of the cGMP dependent kinase (cGKI or PKG), cyclic nucleotide gated (CNG) ion channels, and phosphodiesterases of cGMP dependent nucleotides [2, 4].

The possible neurotoxic effects of $NO^{\cdot}$ include the following,

1) Deamination of bases, which induces DNA damage, activation of the enzyme poly-(ADP-ribose)-synthase, depletion of adenosine-triphosphate (ATP) and nicotinamide-adenine-dinucleotide (NAD), leading to cell death [2, 6].

2) Interaction with ferritin to release iron, which activates the oxidative stress processes [2]. Interestingly, metallothioneins 1 and 2 (a family of proteins which bind to physiological or xenobiotic heavy metals through their thiol group) are able to attenuate NO and $ONOO^{-}$-induced oxidative stress, as indicated by the finding of increased nitrite ion synthesis in metallothionein knockout mice (that develops a progressive nigrostriatal degeneration and shows metallothionein down-regulation) [8].

Table 2. Locations of NO synthase (NOS) in the Nervous System and expression of the different isoforms of NOS (information taken from reference [2]).

A. Central Nervous System.
1) Main locations: microglia and some neuronal populations in the brain such as basket and granule cells of the cerebellum, pedunculopontine nucleus, medium to large aspiny neurons within the striatum, and cerebral cortex (in the striatum and cerebral cortex is colocalized with NADPH-diaphorase, somatostatin and neuropeptide Y).
2) Other locations: ganglionic cells of the adrenal medulla, supraoptic and paraventricular nuclei of the hypothalamus, olfactory bulb, colliculus, dentate gyrus of the hippocampus, stria terminalis, Calleja's islands, and Broca's diagonal band.
B. Peripheral Nervous system (colocalized with NADPH-diaphorase).

3) Modification of the activity of enzymes containing iron-sulfur centers, such as the complexes I and II of the mitochondrial respiratory chain and aconitase, and inhibition of citochrome c oxidase or complex IV (leading to oxidative stress) [6, 9, 10] and ribonucleotide-reductase, that reduces DNA synthesis [2].

4) S-nytrosilation of proteins, that is, a covalent addition of a NO group to a cysteine thiol/sulphydryl group [2-4, 11]. This reaction causes modification of the activity of many enzymes, including activation of plasminogen tissue activator and G-proteins, and inhibition of catepsine B, aldolase, p-glutamyl-cystenil-synthase, alcohol- and aldehyde-dehydro-genase, and glyceraldehyde-3-phosphate-dehydrogenase [2]. S-nytrosilation of specific neuronal proteins (such as alpha-synuclein, parkin and beta-amyloid), leads to protein misfolding, endothelial reticulum stress and mitochondrial impairment [3].

5) Participation in inflammatory phenomena in the Central Nervous System. The cellular response to tissue damage produces microglial activation (and, to a lesser extent, astrocyte activation) with release of proinflammatory factors (cytokines, proteases, and free radicals including reactive oxygen and nitrogen species such as NO), which can lead (in case of persistence) to apoptotic neuronal death [4].

6) Participation in excitotoxicity processes in the Central Nervous System. Activation of glutamatergic NMDA receptors by glutamate is a main stimulus to release NO. NO can have a dual action blocking the reuptake of glutamate (therefore inducing the activation of NMDA receptor and increasing neuronal death) or blocking the NMDA receptor itself (acting as neuroprotective) [2-4]. In addition, treatment of mesencephalic cultures with the glutamate agonist alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) induces neuronal death through nuclear translocation of NF kappa B, and transcriptional activation of the oncogene p53, which is partially prevented by NOS inhibitors [12].

Interaction between Nitric Oxide and Dopamine

Since the main neurochemical feature of PD is the depletion of dopamine (DA) in the nigrostriatal system, interaction between NO and DA could be important in the pathogenic mechanisms of this disease.

Mitkovski *et al.* [13] suggested that communication between nitrergic and dopaminergic systems should play an important role in the control of nigrostriatal pathway, and demonstrated, using an immunohistochemical method and laser scanning microscopy, colocalization of neurons positive for NOS1 and tyrosine-hydroxylase (the enzymes responsible of NO and DA synthesis, respectively) in the nigrostriatal pathway.

Mesencephalic dopaminergic neurons in culture are relatively resistant to NO induced citotoxicity, being this fact likely related with inhibition of conversion of NO to $ONOO^-$ possibly due to suppression of O_2^- production [14]. Moreover, administration of NO donors to primary midbrain cultures has neurotrophic effects, but following depletion of glutathione (GSH) through administration of inhibitors of GSH synthesis, administration of NO donors causes a high neurotoxic effect, which is partially prevented by sGC and PKG inhibitors and, to a lesser extent, by ascorbate [15]. Depletion of GSH in dopaminergic PC12 cell lines causes inhibition of mitochondrial complex I related with direct effect of NO or through inhibition of the tricarboxilic acid (TCA) cycle enzyme alpha-ketoglutarate dehydrogenase (KDGH) [16].

The byproduct of oxidation of DA, DA-quinone, is toxic against dopaminergic neurons by binding and

inactivating proteins through the thiol/sulphydryl group of cysteine. Both NO, $ONOO^-$ and nitrites have shown ability to oxidize DA in a concentration- and pH-dependent manner [17, 18], and high concentrations of NO induce nitrosation and subsequent nitration of DA [19].

Despite DA alone does not elicit mitochondrial damage in dopaminergic PC12 cell lines, this amine can potentiate the effects of NO (irreversible damage of mitochondrial complex I) at the threshold toxic level, being this effect partially prevented by depletion of DA from the storage vesicles [18]. A similar finding was reported with the interaction of dihidroxyphenylacetic acid (DOPAC), one of the main DA metabolites, and NO, which inhibits synergistically cytochrome c oxidase (mitochondrial complex IV) in brain mitochondria of rats, being this effect decreased by metmyoglobin (a ligand for NO and nitroxyl anion) and enhanced by ferricyanide (a reductant of NO which produces nitroxyl anion) [20].

NITRIC OXIDE IN PATIENTS WITH PARKINSON'S DISEASE

Brain

Histoenzymology using the NADPH-diaphorase activity of NOS has shown a significant loss of NOS containing neurons in the putamen of PD patients in a study [21], while in other neurons containing NOS and NADPH-diaphorase within the stritatum were relatively spared, despite the presence of morphological changes, in PD patients [22]. Hunot *et al.* [23] showed increase in NADPH-diaphorase glial cells in the dopaminergic cell groups affected by neuronal loss in the PD mesencephalon, and a greater neuronal loss for NADPH-diaphorase-negative than for those positive for this staining, supporting the relative sparing of NADPH-diaphorase positive neurons.

NOS mRNA expression has been found significantly increased in the dorsal two-thirds of the subthalamic nucleus, and in the medial medullary lamina of the *globus pallidus*, and decreased in the *putamen* of PD patients as compared with controls [24]. The expression of NOS2 has been found up-regulated in the PD *substantia nigra* and similar to that of controls in the *striatum* of PD patients [25].

Nytrosil complexes have been detected in the *substantia nigra* of PD patients (most of them related with haem-NO) at a higher concentrations than in controls [26]. Nitrotyrosine immunorreactivity (indicative of nitration of the tyrosine residues of proteins) has been found in the core of the Lewy bodies within melanized neurons and in amorphous deposits associated with both intact and degenerating neurons in the PD brain [27].

S-nitrosylation of parkin (E3 ubiquitin ligase involved in the ubiquitination of proteins important in the survival of dopaminergic neurons, mutations of its encoding gene are related with familial PD) have been demonstrated in the brain of PD patients [28]. Interestingly, S-nitrosylation of parkin decreases its activity as a repressor of p53 gene expression, leading to an increase in p53 protein levels which induces apoptosis and neuronal death in experimental models of PD, which is partially prevented by pretreatment with NOS inhibitors [29].

Tsang *et al.* [30] described S- nitrosylation of X-linked inhibitors of apoptosis (XIAP) both in PD brain, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice, and in Human Embryonic Kidney 293 (HEK293) cells in culture. These proteins regulate cell survival through binding to caspases. S-nitrosylation of XIAP reduces their anticaspase-3 action, and therefore, their antiapoptotic function.

Uehara *et al.* [31] reported S-nitrosylation of protein-disulphide isomerase (PDI) in the brain of patients with PD and AD. S-nitrosylation inhibits the enzymatic activity of PDI, leading to the accumulation of polyubiquitinated proteins and to activation of unfolded protein response, which results in a decrease in the PDI-mediated attenuation of neuronal cell death related with endoplasmic reticulum stress, misfolded protein or proteasome inhibition.

Fang *et al.* [32] described increased S-nitrosylation of peroxiredoxin 2 (Prx2, a peroxidase that reduce intracellular peroxides, and oxidative stress, with the thioredoxin system as the electron donor) in the human PD brain.

Cerebrospinal Fluid

The results of studies on cerebrospinal fluid concentrations of NO markers (nitrites, nitrates, nitrotyrosine-containing proteins) in PD patients compared with controls have been recently reviewed [33]. Most studies found normal levels nitrites [34-37] and nitrates [35, 38], with the exception of one study reporting increased levels of both nitrites and nitrates [39], other describing decreased nitrates [34], and other increased nitrites [40]. In addition, two studies reported increased nitrotyrosine-containing proteins [41, 42].

Blood and Saliva

Plasma levels of nitrate [38, 43, 44] and nitrite [44] have been reported to be similar in PD patients than those of controls, including PD patients carrying *parkin* gene mutations [45]. Tuncel *et al.* [46] reported higher serum nitrite levels in PD patients than in controls, while other authors found increases serum or plasma nitrate+nitrite [47, 48] and peroxynitrite levels in PD patients [47], and a positive correlation between these parameters and the Unified PD Rating Scale score in the PD group [47].

Gatto *et al.* described increased basal and induced NO production [49, 50], increased protein tyrosine nitration [50], and increased nNOS mRNA expression [50] in neutrophils. This group also reported increased production of NO by neutrophils of healthy controls after incubation with plasma of drug-naïve PD patients or levodopa-treated patients in comparison with those incubated with plasma from healthy controls, therefore suggesting the presence of plasma circulating factors in PD patients as the responsible of this increased NO production [51].

Finally, Huskić *et al.* [52] described decreased salivary nitrite concentration in PD patients as compared with controls.

NOS Genes and Risk for PD

Lo *et al.* [53] described association between the rs72309850 (2bpSTR) polymorphism in the 5' flanking region of the *NOS1* gene and the risk for PD, a finding that was replicated in Caucasian North American patients [54].

Levecque *et al.* [55] reported increased risk for PD in carriers of the minor allele of the rs1047735 SNP in exon 18 of the *NOS1* gene, a finding that, although not confirmed by Hague *et al.* [56], was replicated in other study involving a large sample of families with PD (1065 cases and 1180 relatives and other controls) [57]. Levecque *et al.* [55] reported increased risk for PD in carriers of the minor allele of the rs2682826 SNP in exon 29 of *NOS1* gene as well, but this finding was not replicated in two further studies [56, 58]. Hancock *et al.* [57] reported significant association with PD for 8 out of 27 SNPs in the *NOS1* gene in earlier-onset families with sporadic PD (rs3782218, rs11068447, rs7295972, rs2293052, rs12829185, rs1047735, rs3741475, and rs2682826).

The rs1060826 SNP in the *NOS2* gene has been associated in several studies with decreased risk for PD [55, 56] and with earlier onset of PD [57, 59]. Moreover, in one of these studies this SNP interacted significantly with smoking habits, showing an inverse association between smoking and PD risk only among risk allele noncarriers [59]. Hancock *et al.* [57] reported significant association with PD for 7 out of 18 SNPs in the *NOS2* gene in earlier-onset families with sporadic PD (rs2072324, rs944725, rs12944039, rs2248814, rs2297516, rs1060826, and rs2255929). In contrast, two other studies showed lack of association between rs1060826 SNP and PD risk [58, 60].

Regarding *NOS3* gene polymorphisms, Huerta *et al.* [59] described lack of association between the 27 bp variable tandem repeat polymorphism (27bpVNTR, intron 4), and the risk for PD. In addition, Hancock *et al.* [57] did not find association between 5 SNPs in the *NOS3* gene and PD risk.

NITRIC OXIDE IN EXPERIMENTAL MODELS OF PARKINSONISM

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)

Systemic administration of the neurotoxin MPTP causes a severe nigrostriatal dopaminergic damage, resembling that of idiopathic PD, in many experimental models. This neurotoxic effect of MPTP requires its metabolization to the 1-methyl-4-phenylpyridinium ion (MPP+) through the action of mono-amine oxidase B (MAOB). Intrastriatal administration of MPP+ induce nigrostriatal dopaminergic damage as well. There are many evidences suggesting a role of NO in the dopaminergic neurodegeneration induced by MPTP/MPP+,

1) Mice knockout for *NOS1* [61] or for *NOS2* genes [62] show resistance to the neurotoxic effect of MPTP when compared with the respective wild-type littermates. However, lack of NOS1 activity, but not lack of NOS2 activity, attenuated MPTP induced DNA damage [63].

2) Administration of MPTP to mice induces upregulation of NOS2 [62, 64, 65], and increases immunoreactivity for NOS1 in the *substantia nigra* [66], while NOS1 shows no changes in immunoreactivity in the striatum [66], and NOS3 does not show changes in immunoreactivity neither in the striatum [66, 67] or in the *substantia nigra* [66]. The upregulation of NOS2 in the striatum occurs more rapidly in male than in female mice and in young than in aged mice [67], as the increases in NOS1 protein expression occurs more rapidly

in young male than in young female mice [67]. The upregulation of NOS2 induced by MPTP in the mice *substantia nigra* seems to be mediated by P38-mitogen activate protein kinase (P38MAPK) signalling, because inhibitors of P38MAPK protect dopaminergic neurons in this PD model [65].

3) MPTP does not induce significant changes in the morphology, distribution and number of NOS positive cells in the pedunculopontine nucleus of monkeys [68].

4) Pretreatment with NOS1 inhibitors such as 7-nitroindazole (7-NI) protects against MPTP-induced neurotoxicity (striatal DA depletion and loss of tyrosine-hydroxylase neurons in the *substantia nigra*) in mice [61, 69] and in baboons [70]. Despite it was suggested that this action seemed to be unrelated with alterations in the content of MPP+, the active metabolite of MPTP [61], 7-NI also prevents nigrostriatal damage induced by intrastriatal infusion of MPP+ in rats [71].

5) Pretreatment with the NOS1 and NOS3 inhibitor N(G)nitro-L-arginine methyl-esther (L-NAME) decreases the hydroxyl radical ($OH^{\cdot}$) production induced by coadministration of MPP+ and KCl to rat (but it has no effect on $OH^{\cdot}$ production of coadministration of MPP+ with iron or DA), suggesting that this effect should be related with increased NO synthesis [72].

6) Pretreatments with green tea (which contains high levels of (-)-epigallocatechine 3-gallate or EGCG) [73], or with Gingko biloba Pingchan Recipe [74], decrease the expression of NOS1 in the *substantia nigra* and in the striatum and show a neuroprotective effect against MPTP-induced parkinsonism in mice.

7) Pretreatment with carnosine attenuates MPTP-induced neurotoxicity in the striatum of mice by several mechanisms which include decreased expression or NOS2 and decreased nitrite levels [64]. Other antioxidant substances such as the andrographolide-lipoic acid conjugate AL-1 [75] and salidroside [76] attenuate MPTP and MPP+-induced neurotoxicity by multiple mechanisms which included decrease in NO levels,

8) Mynocicline, an inhibitor of microglial activation, prevents nigrostriatal dopaminergic neurodegeneration and formation of nitrotyrosine induced by MPTP both in normal mice and in NOS2-deficient mice [77]. The flavone acacetin, other inhibitor of microglial activation, decreases NOS2 activity and NO production and prevents MPTP-induced neurotoxicity in the nigrostriatal system of mice and MPP+-induced toxicity in primary mesencephalic cultures [78].

9) NO donating nonsteroidal anti-inflammatory drugs attenuate MPTP-induced neurotoxicity through decreasing NOS2 expression and by downregulation of 2-nitrotyrosine [79].

10) Pretreatments with the herbals Bushenhouxue Yin [80] and Tanshinone I (a flavonoid derivative) [81] attenuate MPTP-induced neurotoxicity in mice by reducing microglial activation and concentrations of NO and several cytokines.

11) Pretreatment with the flavonoid derivative silbinin attenuates MPP+-induced neurotoxicity in mice, in a dose-dependent manner, by reducing the increased levels of NOS2 and cytokines such as tumor necrosis factor-alpha (TNF-alpha) and interleukin 1-beta [82].

12) Pretreatment with copper prevents MPP+ induced neurotoxicity and protein nitration in the mice striatum, which is related with a reduction of NOS1 activity [83].

13) Preadministration of sublethal doses of MPP+ to rat mesencephalic dopaminergic cultures enhances NO-induced neurotoxicity [14]. However, despite peroxynitrite donors induce higher protein nitration than MPP+ in organotypic mouse mesencephalon cultures, a significant DA depletion is reached with much lower concentrations of MPP+, and, although the NOS1 and NOS3 inhibitor L-NAME prevents protein nitration induced by MPP+ in this model, it does not prevent MPP+-induced DA depletion [84].

6-Hydroxydopamine (6OHDA)

Stereotactic injection of 6OHDA into the midbrain of rats causes an important loss of dopaminergic neurons in the *substantia nigra* resembling those of PD. Administration of amphetamine to the parkinsonian 6OHDA rat model induces rotations, while treatment with levodopa induces abnormal involuntary movements resembling levodopa-induced dyskinesia in hu-

mans with PD. Several experimental data suggest a role of NO in the 6OHDA induced neurodegeneration,

1) Striatal injection of 6OHDA causes a significant tardive decrease of NOS1 activity [84], and decrease of NOS1 expression which was more marked in animals treated with the NO donor molsidomine according to some reports [86], or increased NOS1 expression according to others [87], and increase in NOS2 expression and in nitrite levels [88]. The number of neurons immunorreactive to NOS1 in the *substantia nigra* has been found decreased by 25% both in 6OHDA lesioned rats treated with vehicle, levodopa or levodopa plus the NO donor molsidomine [86].

2) Pretretament with a NOS1 and NOS3 inhibitor (L-NAME) protects against 6OHDA-induced neurotoxicity [85, 88], blocks amphetamine-induced rotations [85, 88], and attenuates levodopa-induced dyskinesias [89, 90]. The selective NOS2 inhibitor GW274150 also protected against 6OHDA neurotoxicity [91]

3) Pretreatment with NOS1 inhibitors such as 7-nitroindazole (7-NI) reduces 6OHDA-induced neurotoxicity in the nigrostriatal system [87, 92, 93], and attenuates levodopa-induced dyskinesia [89, 94]. The mechanism of attenuation of levodopa-induced dyskinesias by 7-NI seems to be related with reduction in upregulation of NOS1 interneurons in the lateral striatum caused by 6OHDA, together with a reduction in DA and serotonin turnover [94].

4) Pretreatment with green tea polyphenols prevents the neurotoxic effect of 6OHDA, both in SH-SY5Y cells in culture [95] and in the 6OHDA rat model of parkinsonism [96], by counteracting the increase of NO concentrations, the increased overexpression of NOS1 and NOS2, and by decreasing the level of protein-bound nitrotirosine.

5) The morphology, distribution and number of NOS positive cells in the pedunculopontine nucleus [68] and in subthalamic nucleus [86] were similar in 6OHDA lesioned rats and in controls.

Other experimental models of parkinsonism or nigrostriatal degeneration

Systemic administration of ***methamphetamine*** to mice causes nigrostriatal damage, which is prevented by pretreatment with the NOS1 inhibitor 7-NI [97, 98]. Methamphetamine also causes increased expression of NOS and increased concentrations of NO in PC12 cells, which are reversed by coadministration of N-nitro-L-arginine [99].

Lipopolysaccharides (LPS) induce microglial and macrophage activation and cause dopaminergic damage when injected in the striatum [88] or in the *substantia nigra* of mice [100, 101], in several dopaminergic cell lines (MES 23.5 cells) [102], or in dopaminergic neurons in primary mesencephalic cell cultures [102, 103]. LPS causes overexpression of NOS2 [88, 100, 101], and increases NO production [88, 102, 103] and 3-nitrotyrosine formation [100] in these experimental models. Pretreatment with L-NAME prevents amphetamine induced rotations in the mice model [88], and pretreatment with NOS2 inhibitors, such as S-methylisothiourea [100], or L-NAME [101] reduces LPS induced neurotoxicity.

LPS activated astrocytes cause neuronal death and increase the neurotoxic effect of MPP+ or 6OHDA in ventral mesencephalic neuron cultures, being this effect inhibited by the NOS2 inhibitor aminoguanidine and by NO scavengers [104]. Co-cultures of mouse reactive macrophage cells (RAW 264.7) stimulated by LPS and SH-SY5Y cells (as a model for human dopaminergic neurons) show increased NO production, increased peroxynitrite concentrations and nitration of alpha-synuclein within SH-SY5Y cells, leading to dopaminergic cell death, which is partially prevented by a NOS2 inhibitor decreasing the levels of nitrated alpha-synuclein [105].

Kurauchi *et al.* [106], in a study using rat midbrain slices cultures showed that activation of microglia by ***coadministration of LPS and interferon gamma*** (IFN-gamma) caused dopaminergic cell death which was dependent on NO production, and increase in the heme oxygenase 1 (HMOX-1 or HO-1) expression, in the surviving dopaminergic cells. The neurotoxic effect of LPS and IFN-gamma was exacerbated by inhibitors of sGC and of PKG, NO donors and HMOX-1 inhibitors, and was attenuated by cGMP analogs. HMOX-1 expression in the surviving dopaminergic cells was decreased by inhibitors of sGC and of PKG, and increased by cGMP analogs. These data suggest an important role of the NO/cGMP signalling pathway in the induction of HMOX-1 as a protective factor against inflammatory phenomena leading to cell death.

Selective inhibitors of NOS2 have a protective action against selective loss of dopaminergic neurons in mesencephalic cultures induced by ***costimulation with***

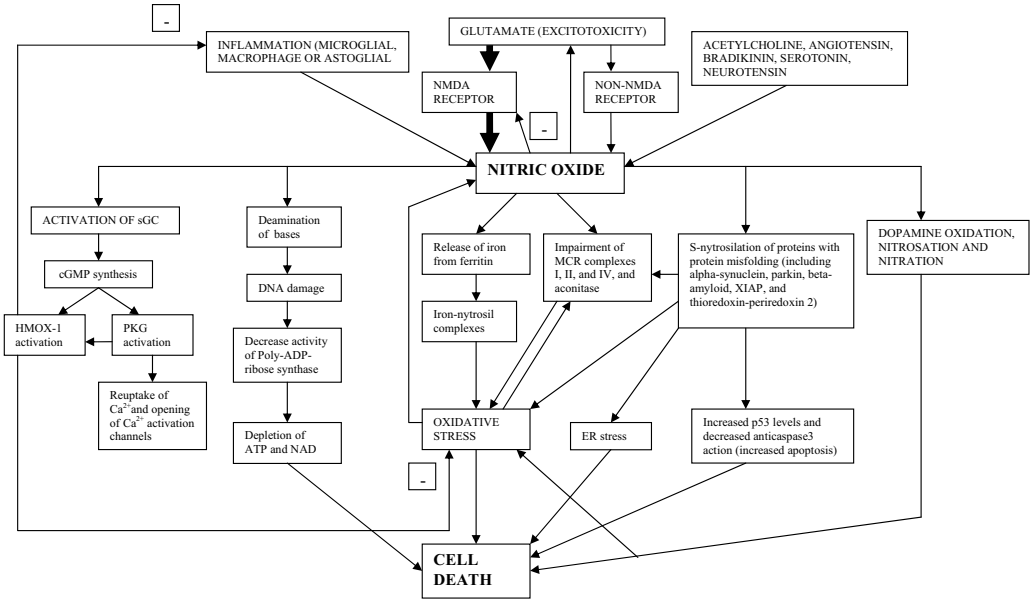

Fig. (2). Schematic representation on the possible role of nitric oxide in the different mechanisms leading to nigral neuronal death in Parkinson's disease.

the protein cluster of differentiation 40 (CD 40) and IFN-gamma [107].

Administration of ***pesticides such as maneb and paraquat*** to mice induces a parkinsonian model. Pretreatment with the NOS2 inhibitor aminoguanidine attenuates significantly the NOS2 mRNA expression, nitrite concentrations, and lipid peroxidation in the striatum induced by these toxins, suggesting a role of NO in their neurotoxic effect [108].

In a model of experimental parkinsonism consisting of ***transgenic rat expressing the human G2019S mutation in the LRRK2 gene*** (this mutation causes autosomal dominant late-onset PD and is the most frequent genetic cause of this disease) it has been shown increased oxidative stress and increased NOS2 expression in the *substantia nigra* and in the striatum, and abnormal morphology of nigral dopaminergic neurons when compared with wild-type rats [109].

Both the chronic and the acute administration of the NOS1 inhibitor 7-NI prevent the development of levodopa-induced dyskinesia or attenuates the established levodopa-induced dyskinesia, without reduction in the therapeutic effect of levodopa, in the ***Pitx3(-/-) aphakia mice*** (a genetic model of parkinsonism), suggesting the role of NO in this complication [110].

The β-amyloid precursor protein (APP, a protein associated with AD) facilitates neuronal export. ***APP(-/-) mice*** develop iron-dependent nigral cell loss resembling neuropathological changes of PD, while APP-overexpressing mice are protected against MPTP neurotoxicity. NO is able to suppress APP translation in mouse MPTP models, and contribute to iron-dependent neurodegeneration in this model [111].

CONCLUSION

There are many evidences arising, both from neuropathological studies in the brain of PD patients and from studies in diverse experimental models of dopaminergic damage, which suggest an important role of NO in the cascade of processes leading to neurodegeneration in this particular disease. Moreover, the fact that non-selective NOS inhibitors and selective NOS1 or NOS3 inhibitors can prevent the neurotoxic effects of dopaminergic toxins such as MPTP, 6OHDA, methamphetamine, and LPS (among others), as it has been shown in many reports mentioned in this review, support the involvement of NO in those effects, although clinical applications of NOS inhibitors have not been developed yet. Data from studies of cerebrospinal fluid or plasma concentrations of NO markers are not conclusive, while increased production of NO by neutrophils has been shown in PD patients. To date, there is not convincing evidence of the contribution of *NOS* genes in the modification of the risk for PD.

Fig. (**2**) represents a temptative diagram of the possible interactions of NO in the neurodegenerative processes of PD, including those with dopamine, iron, and glutamate, its role as proinflammatory factor and in oxidative stress phenomena. In recent years, a special attention has been paid to S-nitrosylation, S-nitr-

ositation and S-nitration of proteins [3, 11] as the most important mechanisms of toxicity of NO. Moreover, S-nitrosylation of certain proteins such as alpha-synuclein and parkin (both involved in familial forms of PD), mitochondrial respiratory chain complexes (a deficiency of complex I activity in the *substantia nigra* is well-established in PD), and beta-amyloid, should have an important relevance in the pathogenesis of PD.

LIST OF ABBREVIATIONS

AD	=	Alzheimers's disease
ADP	=	adenosine-diphosphate
AMPA	=	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	=	adenosine-tryphosphate
CD 40	=	cluster of differentiation 40
cGKI or PKG	=	GMP dependent kinase
cGMP	=	guanosine 3'5'-cyclic monophosphate
CNG	=	cyclic nucleotide gated
DA	=	dopamine
DNA	=	Deoxyrribonucleic acid
DOPAC	=	dihydroxyphenylacetic acid
EDRF	=	endothelium derived relaxing factor
EGCG	=	(-)-epigallocatechine 3-gallate
eNOS	=	endothelial nitric oxide synthase
ER	=	endoplasmic reticulum
FAD	=	flavine-adenine-dinucleotide
FMN	=	flavine mononucleotide
GSH	=	reduced glutathione
HD	=	Huntington's disease
HEK293	=	Human Embryonic Kidney 293
IFN gamma	=	interferon gamma
iNOS	=	inducible nitric oxide synthase
KDGH	=	alpha-ketoglutarate dehydrogenase
L-NAME	=	N(G)nitro-L-arginine methyl-esther
LPS	=	lipopolysaccharide
MAOB	=	mono-amine oxidase B
MPP+	=	1-methyl-4-phenylpyridinium ion
MPTP	=	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	=	mensagere ribonucleic acid
MS	=	Multiple sclerosis
NAD	=	nicotinamide-adenine-dinucleotide
NADPH	=	nicotinamide-adenine-dinucleotide-phosphate
7-NI	=	7-nitroindazole
NMDA	=	N-metyhl-D-aspartate
NO	=	Nitric oxide
$NO^{\cdot}$	=	NO free radical
NO^{+}	=	Nitrosonium cation
NO^{-}	=	Nitrosyl anion
NOS	=	nitric oxide synthase
nNOS	=	neuronal nitric oxide synthase
O_2^{-}	=	Superoxide anion
6OHDA	=	6-hydroxydopamine
$OONO^{-}$	=	Peroxynitrite anion
P38MAPK	=	P38-mitogen activate protein kinase
PD	=	Parkinson's disase
PDI	=	protein-disulphide isomerase
Prx2	=	peroxiredoxin 2
sGC	=	soluble guanylyl cyclase
TCA	=	tricarboxilic acid
TNF	=	alpha tumor necrosis factor alpha

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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