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Brain mitochondrial dysfunction and oxidative damage in Parkinson's disease

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Abstract Complex factors contribute to the appearance of Parkinson's disease (PD), but with a constant mitochondrial involvement. There are two interdependent conditions in PD: brain mitochondrial dysfunction and brain mitochondrial oxidative damage. Mitochondrial dysfunction and reduced complex I activity are recognized in *substantia nigra* and in frontal cortex in PD patients. The molecular mechanism involved in the inactivation of complex I is likely accounted by the sum of $ONOO^-$ mediated reactions, reactions with free radical intermediates of the lipid peroxidation process and amine-aldehyde adduction reactions. The inhibitory effects on complex I lead synergistically to denaturation of the protein structure and to further increases of O_2^- and $ONOO^-$ production at the vicinity of complex I. An adaptive response in PD patients has been described with increases in mtNOS activity, mitochondrial mass and mitochondrial biogenesis. Mitochondrial dysfunction in the human frontal cortex is to be considered a factor contributing to impaired cognition in PD.

Keywords Complex I · Frontal cortex mitochondria · Brain mitochondria in PD · mtNOS

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Parkinson's disease

It has been recognized that inherited, metabolic and environmental factors contribute to the appearance of Parkinson's disease (PD) but the pathogenic mechanism remains to be elucidated. Parkinson's disease is an old-age neurodegenerative disease with a prevalence of 0.3% in the entire population, affecting more than 1% of the humans over 60 years of age (de Lau and Breteler 2006). Parkinson's disease is clinically manifested by resting tremor, slowness of movements, rigidity and postural instability and it is pathologically defined by loss of neurons in the *substantia nigra, striatum* body and brain cortex and by the presence of cytoplasmic protein inclusions named Lewy bodies and neurites (Forno 1996; Jellinger and Mizuno 2003).

Mitochondria and Parkinson's disease

There is considerable evidence for a mitochondrial involvement in sporadic PD. Multiple genes in which mutations or polymorphisms increase the risk of PD are linked to mitochondrial functions. Experimental data *in vivo* and *ex vivo* indicate that PD is associated to two interdependent conditions of brain mitochondria: (a) mitochondrial dysfunction; and (b) mitochondrial oxidative damage. Several studies have shown mitochondrial dysfunction and reduced activity of mitochondrial complex I in *substantia nigra* (Schapira et al. 1990b; Schapira et al. 1990a; Schapira 2008) and in frontal cortex (Navarro et al. 2009) in PD patients. Moreover, similar mitochondrial complex I dysfunctions were reported in skeletal muscle and platelets of PD patients (Mann et al. 1992). This condition of complex I impairment is likely to be of pathogenic importance

because intoxication of experimental animals with inhibitors of complex I (rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its metabolite 1-methyl-4-phenylpyridinium (MPP+) (Bougria et al. 1995; Gomez et al. 2007) reproduce the clinical symptoms of PD in human subjects.

Mitochondrial complex I

Mitochondrial complex I (NADH-UQ reductase) catalyzes electron transfer from NADH to ubiquinone and is the main molecular pathway to connect the tricarboxylic acid cycle, the coenzyme NADH and the mitochondrial respiratory chain. Complex I is a supra-molecular protein complex composed of about 40 units and contains FMN and iron–sulphur centers. Seven polypeptides of complex I are encoded by mitochondrial genes (ND 1, 2, 3, 4, 4 L, 5, and 6). Main complex I components include a 54 kDa flavoprotein, 24, 75 and 49 kDa proteins, and the proteins TYKY and PSST that are bound to the inner membrane and that transfer electrons to UQ (Walker 1992). Two complex I-linked UQ-pools have been detected (Raha and Robinson 2000). Non-covalent hydrophobic bonds are important in keeping together the whole structure of complex I; low concentrations of detergents, natural and synthetic steroids (Boveris and Stoppani 1971) and hydrophobic pesticides (rotenone and pyridaben; Gomez et al. 2007) are effective in disrupting intra-complex I polypeptide hydrophobic bonds and in inhibiting complex I electron transfer activity. The rate of electron transfer in complex I is relatively high; NADH oxidation proceeds at 250–500 nmol/min.mg protein, as compared with the rate of electron transfer of UQH_2-cytochrome *c* reductase, of 100–150 nmol/min.mg protein (Brown and Borutaite 2004). In addition to complex III, complex I produces by autooxidation with molecular O_2 significant amounts of O_2^-. It is understood that in complex I the main reaction is the autoxidation of the flavin semiquinone FMNH·, with a minor contribution the semiquinone UQH· that is bound to the complex I-linked UQ-pools (Boveris and Cadenas 2000; Turrens 2003). This rate of O_2^- production by complex I is increased by inhibition of electron transfer with rotenone (Boveris and Chance 1973) or by complex I dysfunction (Hensley et al. 2000).

Mitochondrial complex I is particularly sensitive in terms of inhibition and inactivation to oxidants, oxygen free radicals and reactive nitrogen species. This special characteristic is frequently referred as the "complex I syndrome", with the symptoms of reduced state 3 (with ADP) mitochondrial respiration with malate-glutamate as substrate and of diminished complex I activity. This "complex I syndrome" has been observed in PD and in other neurodegenerative diseases (Schapira et al. 1990b; Schapira et al. 1990a; Schapira 2008; Carreras et al. 2004; Navarro et al. 2009), as well as in aging (Boveris and Navarro 2008) and in ischemia-reperfusion (Gonzalez-Flecha et al. 1993). The molecular mechanism involved in the inactivation of complex I is likely accounted by the sum of $ONOO^-$ mediated reactions, reactions with the free radicals intermediates of the lipid peroxidation process (R· and ROO·) and amine-aldehyde adduction reactions. It is now understood that the three process above mentioned that alter the native non-covalent polypeptide interactions promote synergistically protein damage and inactivation by shifting the non-covalent bonds to covalent cross linking (Liu et al. 2003).

Nitric oxide and peroxynitrite as complex I inhibitors

Both, NO and ONOO- have been reported as direct inhibitors of complex I. It has been claimed that NO inhibits mitochondrial complex I activity by S-nitrosylation and Fe-nitrosation (Brown and Borutaite 2004). On the other hand, irreversible inhibition of brain mitochondrial complex I by $ONOO^-$ was reported (Riobo et al. 2001). The current ideas are that the chronic effects of NO are explained by $ONOO^-$ increased levels in the mitochondrial matrix. It is considered that the anion $ONOO^-$ is confined to the mitochondrial matrix, since it is charged and there is no recognized transporter. Peroxynitrite is generated in the mitochondrial matrix by the termination reaction of the two free radicals O_2^- and NO. Superoxide radical is produced physiologically in mitochondria by the autoxidation reactions of FMN· and UQH· at a rate of 0.80–0.90 nmol O_2^-/min.mg protein resulting in a steady state concentration of 0.1–0.2 nM in the mitochondrial matrix (Boveris and Cadenas 2000; Boveris et al. 2006; Valdez et al. 2006). In the same physiologic conditions and by the enzymatic reaction of mitochondrial nitric oxide synthase (mtNOS), NO is produced at a rate of 1.0–1.4 nmol NO/ min.mg protein and kept at a steady state level of 200–350 nM in the mitochondrial matrix (Boveris et al. 2006; Valdez et al. 2006). Both radicals react between them in a diffusion controlled reaction (k=1.9×$10^{10}M^{-1}s^{-1}$) and generate $ONOO^-$ at a rate of 0.38 μM/sec in the mitochondrial matrix or 0.92 nmol/min.mg protein (Valdez et al. 2000). In this estimation the contribution of cytosolic NO has not been considered. Peroxynitrite is normally reduced by the mitochondrial reductants $NADH_2$, UQH_2 and GSH and kept at intramitochondrial steady state level of 2–5 nM (Valdez et al. 2000). When this level is increased in excess, up to 25–40 nM, it leads to tyrosine nitration, protein oxidation and damage to iron sulfur centers with sustained complex I inhibition and increased generation of O_2^- by complex I.

The available evidence indicate that increased mitochondrial matrix NO levels, secondary to over-expression or mtNOS activation or to NO diffusion from cytoslic NOS, leads to complex I inhibition and to increased production of O_2^- and to the turning of normal cell signaling by H_2O_2 and NO to $ONOO^-$-initiated apoptotic signaling. The normal signaling role of NO and H_2O_2 is supported by the observation that the 144 kDa brain mtNOS increases in pre- and post-natal periods in rats in parallel to brain and cerebellum development (Riobo et al. 2002).

In this context, the transcriptional regulation of two brain NOS, the cytosolic nNOS and the mitochondrial mtNOS are particularly relevant. A bipolar and complementary distribution of NOS activities in the cell has been proposed; one in mitochondria (mtNOS) and the other one (nNOS or eNOS) in the cytosol (Navarro and Boveris 2008). There are reports of over-expression of nNOS in the brain of patients with PD (Eve et al. 1998) and of increased levels of cytosolic nNOS in aging rats (Lam et al. 2009). In the latter case, the proteomic analysis of nitrated proteins in aged rats showed an almost specific nitration of the F_1-ATPase at Tyr^{269}, explained by the higher level of ONOO- in the mitochondrial matrix as compared with the cytosol (Lam et al. 2009).

The observed nNOS over-expression and the presence of 3-nitrotyrosine in circulating neutrophils from PD patients, suggests a generalized deregulation of the nNOS gene in PD (Gatto et al. 2000). The role of increased levels of NO in PD gained significance by the finding of 3-nitrotyrosine in the core of Lewy bodies, the pathological hallmark of PD (Good et al. 1998). The presence of 3-nitrotyrosine, the "footprint" of $ONOO^-$ high levels, was observed in brain mitochondria of rats with experimental PD induced by MPTP and its metabolite MPP^+, which was prevented by previous administration of 7-nitroindazole, a relatively specific nNOS inhibitor. Moreover, nNOS-gene deficient mice are more resistant to the toxic effects of MPTP than wild-type animals. In agreement, in SH-SY5Y neuroblastoma cells MPTP and MPP^+ increase the mitochondrial production of NO, suggesting an activating effect on mtNOS that is associated to Bax increase, release of cytochrome c and caspase activation (Dennis and Bennett, Jr. 2003). In this condition, the oxidative stress induced by complex I inhibition also biphasically activates the pro-apoptotic stress-activated factor c-Jun N-terminal kinase (JNK) and the transcription factor NF-κB (Cassarino et al. 2000).

The inhibitory action of $ONOO^-$ on complex I leads to denaturation of the protein structure and further increases of O_2^- and $ONOO^-$ production at the vicinity of complex I itself tending to the perpetuity of this metabolic abnormality (Fig. 1).

Dopaminergic neurons and α-synucleinopathies

It is not defined why *substantia nigra* is particularly vulnerable to the NO toxic effects that occur in PD and

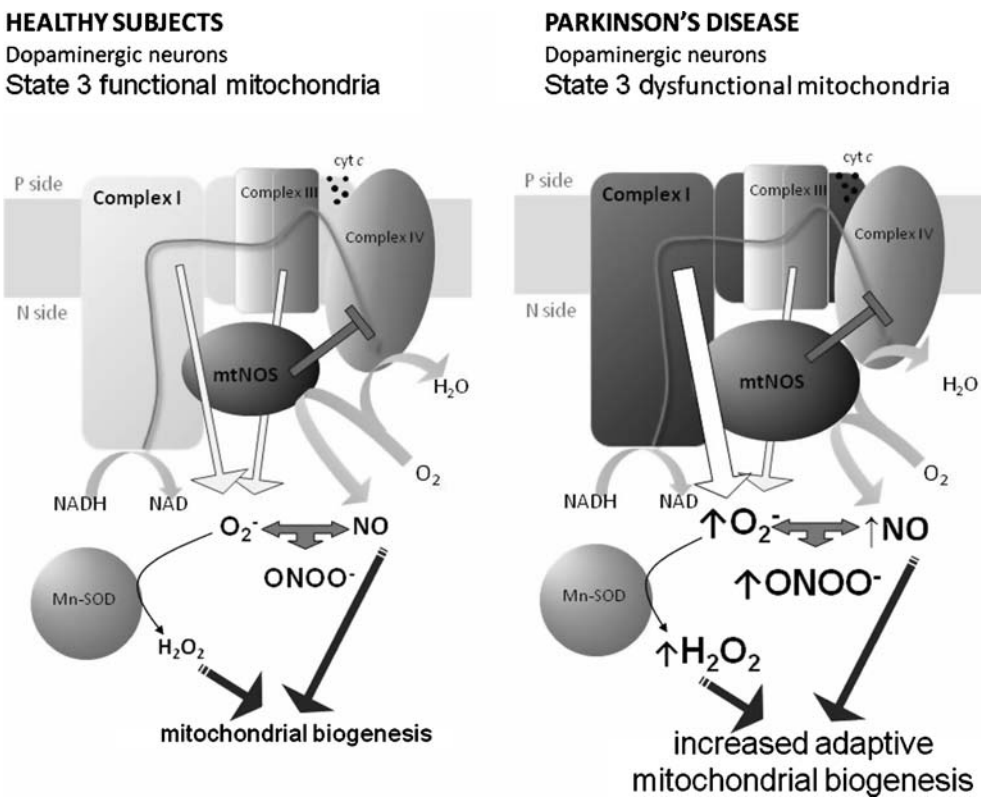

Fig. 1 Scheme of the changes in mitochondrial O_2^-, H_2O_2 and NO metabolism in Parkinson's disease. The normal levels of the three mitochondrial metabolites are increased in PD. Superoxide radical production is increased by modified complex I, H_2O_2 is increased from its stoichiometric precursor (O_2^-), NO is increased from the adaptive response of mtNOS and $ONOO^-$ is increased (with increased $ONOO^-$ dependent mitochodrial damage) from the increased O_2^- and NO steady state levels

that lead to neurodegeneration of dopaminergic and catecholaminergic cells. *Substantia nigra* has high concentrations of 6-hydroxydopamine that readily reacts with NO ($k=1.5\times10^4$ M^{-1} s^{-1}) leading to the formation of dopamine semiquinone and increasing intramitochondrial and intracellular O_2^- and $ONOO^-$ formation (Riobo et al. 2001).

Recent studies have showed that α -synuclein is one of the major building blocks in Lewy bodies (Ischiropoulos 2009). Interestingly, it is apparent that the majority of the Lewy bodies and protein inclusions contain nitrated and oxidized α-synuclein, indicating that oxidative processes participate in the formation of these inclusions. In addition, a defective transfer of proteins to ubiquitin is the result of nitration or nitrosylation of ubiquitin E3-liases. In the proteasome, mutations of the E3-liase parkin explain the appearance of juvenile PD with two associated consequences. First, in the presence of mutated parkins, NOS is less degraded by less active proteasomes. Second, nitrosylation or nitration of parkin may starts or aggravates the process. Finally, NO in excess damages mitochondria, inhibits complex I activity and promotes nitration and oxidation of proteins that are less degraded by less operative proteasomes.

Although the pathogenesis of PD and α-synucleinopathies is complex and involves multiple factors, there is an almost constant association with an impaired function of the mitochondrial respiratory chain and the appearance of abnormal mitochondrial proteins (Kwong et al. 2006). The concept that free-radicals, lipid peroxidation and oxidative damage were involved in the neuronal abnormalities of PD (Adams and Odunze 1991) was simultaneous with the recognition of complex I dysfunction in PD (Mizuno et al. 1989; Schapira et al. 1989). An elevated level of oxidized mitochondrial proteins was reported in α-synuclein transgenic mice (Poon et al. 2005). Impaired electron transfer and deficiencies in the expression of the subunits of complex I have been reported in the *substantia nigra* in PD (Mizuno et al. 1989; Schapira et al. 1989). Complex I subunits with oxidative damage, functionally impaired and misassembled, have been reported in PD brains (Keeney et al. 2006). Moreover, increased mtDNA deletions were recognized in nigral neurons in PD (Bender et al. 2006).

Complex I syndrome in the frontal cortex of PD patients

Recently, moderate and marked impairment of tissue respiration, state 3 mitochondrial respiration with malate-glutamate as substrate and complex I decreased activity, associated with oxidative damage were determined in frozen samples of frontal cortex (area 8) in PD patients in comparison to age-matched healthy controls (Navarro et al. 2009) (Table 1). The mitochondrial impairment observed in frontal cortex in PD is properly described as a reduced frontal cortex respiration, with marked decrease in ccomplex I activity, associated with oxidative damage, the latter determined by the increased content of phospholipid and protein oxidation products. In short, human cortex mitochondrial dysfunction in PD is now added to the classical recognition of mitochondrial dysfunction in *substantia nigra*, which was early considered as specific (Schapira et al. 1990a).

Table 1 Human frontal cortex oxygen uptake, mitochondrial respiration and other properties of human frontal cortex mitochondria in healthy controls and in Parkinson's disease patients

	Healthy controls	Parkinson disease
Tissue oxygen uptake (ng-at O/min.g brain)	386±31 (100%)	323±16* (83%)
Mitochondrial oxygen uptake. Substrate malate-glutamate + ADP (ng-at O/min.mg protein)	35±2 (100%)	23±2** (66%)
Complex I activity (as NADH-cytochrome c reductase, nmol cyt c reduced/min.mg protein)	396±24 (100%)	162±14** (41%)
Protein oxidation (as protein carbonyls, nmol/mg protein)	25±2 (100%)	37±4* (148%)
Phospholipid oxidation (as TBARS, nmol/mg protein)	0.99±0.03 (100%)	1.22±0.04**(123%)
mtNOS activity (nmol NO/min.mg protein)	4.0±0.4 (100%)	7.0±0.5**(175%)
Mitochondrial mass (mg protein/g frontal cortex)	7.9±0.4 (100%)	11.8±0.4**(149%)
Total cytochrome content (%)	100%	145% **

Data from Navarro et al. (2009). *$p<0.05$ and **$p<0.01$ for Parkinson's disease as compared with healthy controls

The markedly higher mtNOS activity observed in human frontal cortex (4.0–7.0 nmol NO/min.mg protein; (Navarro et al. 2009) in comparison with the mtNOS activities in mouse and rat whole brain brain and frontal cortex (0.64–0.67 nmol NO/min.mg protein (Navarro et al. 2008) set the basis for the speculation that this level of human brain mtNOS expression corresponds to an adaptive response of the highly evolved human brain biology, a biology that makes to the human in extraordinary capacity for homeostatic maintenance and longevity, as compared with other mammals. Cutler and co-workers introduced the concept that the Cu,Zn-SOD and Mn-SOD activities of various organs, evolved in mammalian organs as determinants of life span (Tolmasoff et al. 1980; Cutler 1991). In the same way of thinking, it is reasonable to consider that the human brain evolved as a long-living species organ that acquired an innate and adaptive resistance to oxidative damage as compared with other mammals.

The mitochondrial adaptive response of mtNOS in PD patients is speculated as an increased enzyme activity that supports an increased mitochondrial biosynthesis in order to provide an increased energy supply. Nitric oxide signaling for mitochondrial biogenesis and turnover is a recent concept in cell biology. The NO-dependent pathway of mitochondrial biogenesis includes activation of guanylate cyclase, increased levels of cGMP, and activation of a series of transcription factors, such as PPAR-GC-1α, nuclear respiratory factors (NRF-1 and NRF-2) and mitochondrial transcription factor A. This mechanism is supported by observations in brain, kidney, liver, heart and muscle (Nisoli et al. 2004) and ovary (Navarro et al. 2005).

A 49% increase in the mitochondrial mass of human brain cortex was observed in PD patients (Navarro et al. 2009), understood as a partial compensation for the respiratory impairment with a presumable shortage in ATP and energy supply in the PD patients. The decrease in maximal (state 3) respiratory activity is estimated in the range of 34 to 59% (considering the 34% reduced mitochondrial O_2 uptake with malate-glutamate and the 59% decreased complex I activity). It seems that the adaptive response is about to provide the same energy production as in the state of health.

The current views on PD consider that this disease is not only characterized by *substantia nigra* dysfunction but that it also involves the frontal cortex with a cognitive decline at the early stages of parkinsonism (McNamara et al. 2007). Oxidative and nitrosative damage and mitochondrial dysfunction in the human frontal cortex are to be considered factors leading to impaired cognition in PD.

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