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# Inhibition of neuronal mitochondrial complex I or lysosomal glucocerebrosidase is associated with increased dopamine and serotonin turnover

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disorder caused by loss of dopaminergic and serotoninergic signalling. A number of pathogenic mechanisms have been implicated including loss of mitochondrial function at the level of complex I, and lysosomal metabolism at the level of lysosomal glucocerebrosidase (GBA1). In order to investigate further the potential involvement of complex I and GBA1 in PD, we assessed the impact of loss of respective enzyme activities upon dopamine and serotonin turnover. Using SH-SY5Y cells, complex I deficiency was modelled by using rotenone whilst GBA1 deficiency was modelled by the use of conduritol B epoxide (CBE). Dopamine, its principal metabolites, and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the extracellular medium were quantified by HPLC. Inhibition of complex I significantly increased extracellular concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HIAA. Comparable results were observed with CBE. These results suggest increased monoamine oxidase activity and provide evidence for involvement of impaired complex I or GBA1 activity in the dopamine/serotonin deficiency seen in PD. Use of extracellular media may also permit relatively rapid assessment of dopamine/serotonin metabolism and permit screening of novel therapeutic agents.



## 1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder of dopaminergic neurons with characteristic symptoms that include tremor, rigidity and bradykinesia. Despite the considerable research that has been carried out in this field, the primary cause of PD is still unknown. Consequently, current therapies provide a temporary symptomatic relief by aiming to increase dopamine availability. These treatments vary depending on the stage of the disease, but gold standard treatment for PD is the dopamine precursor L-dihydroxyphenylalanine (L-DOPA) along with inhibitors of the dopamine degradation enzymes (Birkmayer et al., 1975).

Concerning putative mechanisms, loss of brain mitochondrial complex I activity has been reported in patients with PD (Schapira et al., 1990). Observations relating to the parkinsonian features associated with exposure to complex I inhibitors, such as rotenone and MPP+, provide credence for deficiency of complex I being a factor in PD. (Dauer and Przedborski, 2003). With regards to the consequences of reduced complex I activity, a number of mechanisms have been proposed, including reduced ATP formation and oxidative stress.

Neuronal complex I activity appears to exert particular control over mitochondrial ATP formation and losses of activity comparable to those seen in PD can be expected to lead to compromised brain

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**Abbreviations**

| | |
|---|---|
| 3-MT | 3-methoxytyramine |
| 3-OMD | 3-*O*-methyldopa |
| 5-HIAA | 5-hydroxyindoleacetic acid |
| 5-HTP | 5-hydroxytryptophan |
| AADC | aromatic amino acid decarboxylase |
| ALDH | aldehyde dehydrogenase |
| BH4 | tetrahydrobiopterin |
| CBE | conduritol B-epoxide |
| COMT | catechol-*O*-methyl transferase |
| DAT | dopamine transporter |
| DOPAC | 3,4-dihydroxyphenylacetic acid |
| ETC | electron transport chain |
| GBA1 | lysosomal glucocerebrosidase |
| GSH | reduced glutathione |
| HPLC | high performance liquid chromatography |
| HVA | homovanillic acid |
| L-DOPA | L-dihydroxyphenylalanine |
| MAO | monoamine oxidase |
| PD | Parkinson's disease |
| PLP | pyridoxal phosphate |
| TH | tyrosine hydroxylase |
| TPH | tryptophan hydroxylase |
| VMAT2 | vesicular monoamine transporter 2 |

energy metabolism (Davey and Clark, 1996). Furthermore, in the presence of oxidative stress, the magnitude of complex I loss required to inhibit ATP generation is significantly less (Davey et al., 1998). Oxidative stress occurs as a result of excessive generation of oxidising molecules, such as reactive oxygen and nitrogen species, and/or loss of antioxidant capacity for scavengers such as alpha-tocopherol, ascorbate and reduced glutathione (GSH; Barker et al., 1996; Bolanos et al., 1995; Riederer et al., 1989). Inhibition of the mitochondrial respiratory chain is associated with increased generation of reactive oxygen species, a situation that appears to precede impairment of energy metabolism (Jacobson et al., 2005).

With regards to antioxidant status, decreased GSH levels have been reported in the brain of patients with PD (Perry et al., 1982; Sian et al., 1994). Furthermore, comparable losses have been reported in patients deemed to have pre-symptomatic PD (Jenner et al., 1992). Although GSH levels in the pre-PD patients are decreased to comparable level to those seen in PD, it is of note that complex I activity was not decreased (Jenner et al., 1992). This raises the possibility that GSH loss precedes and contributes to the loss of complex I activity, a hypothesis that is supported by a number of observations in both cellular and animal models (Barker et al., 1996; Bolanos et al., 1996; Heales et al., 1994, 2011).

Another potential mechanism implicated in PD pathogenesis is failure of lysosomal autophagy (Lynch-Day et al., 2012) leading to impaired protein processing, e.g. alpha-synuclein and formation of Lewy bodies that are characteristic of PD (Beyer, 2007). In post mitotic neurons, this failure of autophagy may also lead to the accumulation of defective mitochondria and provides a further potential mechanism for the compromised mitochondrial function in PD. Support for lysosomal involvement in PD comes from the study of patients with either homozygous mutations (Gaucher disease) or heterozygous mutations in lysosomal glucocerebrosidase (GBA1). Such individuals have a significantly increased risk of developing PD (Neumann et al., 2009). A number of mechanisms have been proposed to link the increased risk of PD with impairment of GBA1. Amongst these are aberrant alpha-synuclein processing, oxidative stress and mitochondrial defects including loss of mitochondrial respiratory chain activity (Cleeter et al., 2013; Mazzulli et al., 2011; Osellame et al., 2013; Sidransky and Lopez, 2012). The link between PD and loss of GBA1 activity is further evidenced by reports of altered dopamine metabolites in CSF of patients with Gaucher/PD (Alonso-Canovas et al., 2010; Machaczka et al., 2012). In such studies, compromised serotonin metabolism was also implicated.

In view of the fact that loss of dopaminergic neurons is the ultimate consequence for all the possible mechanisms considered in PD, its metabolism will be briefly reviewed. Serotonin metabolism will also be considered, due to the possible involvement of this neurotransmitter in PD (Olivola et al., 2014) and the clear overlap between dopamine and serotonin metabolism.

Synthesis of dopamine commences with the transformation of the amino acid L-tyrosine into L-DOPA by the tetrahydrobiopterin (BH4)-dependent tyrosine hydroxylase (TH). This is then converted by aromatic amino acid decarboxylase (AADC), a pyridoxal phosphate (PLP) requiring enzyme, to dopamine (Fig. 1). L-DOPA that is not metabolised via AADC can be converted to 3-*O*-methyldopa (3-OMD) via catechol-*O*-methyl transferase (COMT) (Fig. 1). As dopamine is not stable at physiological pH, it is internalised and stored in synaptic vesicles by vesicular monoamine transporter 2 (VMAT2; Chaudhry et al., 2008). This transporter uses a proton gradient generated by the vacuolar-type ATPase proton pump. In these vesicles the pH is two units lower than in the cytosol, so dopamine does not spontaneously oxidise (Guillot and Miller, 2009). It has been proposed that TH, AADC and VMAT2 interact forming a complex to make dopamine internalisation in vesicles as efficient as possible (Cartier et al., 2010). When acting as a neurotransmitter, dopamine is released into the synaptic cleft and binds to receptors. After its release, dopamine is removed from the synaptic cleft by the pre-synaptic neuronal terminal cells which capture the dopamine via the dopamine transporter (DAT; reviewed by Eriksen et al., 2010). The internalised dopamine can be either recycled or degraded. For recycling, dopamine is sequestered into synaptic vesicles by VMAT2. The non-recycled neurotransmitter is further metabolised via two parallel pathways. In the first route, monoamine oxidase (MAO) and aldehyde dehydrogenase (ALDH) metabolise dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC). Then, DOPAC is subsequently converted to homovanillic acid (HVA) by COMT. In the second route, COMT first transforms dopamine to 3-methoxytyramine (3-MT). HVA is then formed by the consecutive action of MAO and ALDH (Fig. 1).

Dopamine metabolism is closely related to serotonin synthesis and degradation, because both pathways share some enzymes (Fig. 1). Serotonin synthesis starts with the oxidation of L-tryptophan by tryptophan hydroxylase (TPH) and the cofactor BH4. This reaction is the rate limiting step in this pathway and produces 5-hydroxytryptophan (5-HTP). 5-HTP is decarboxylated by AADC with PLP as cofactor, synthesising serotonin. As with dopamine, serotonin has to be quickly degraded after its action. To accomplish this, MAO and ALDH transform serotonin into 5-hydroxyindoleacetic acid (5-HIAA), its final degradation metabolite.

In view of the clear link between PD and loss of mitochondrial and lysosomal function, in this study we have examined the effects of loss of complex I or GBA1 upon dopamine and serotonin metabolism. Extracellular media was evaluated to ascertain whether relatively rapid insight into the metabolism of these neurotransmitters could be achieved.