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In silico evidence for glutathione- and iron-related pathogeneses in Parkinson's disease

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ABSTRACT

Post-mortem analyses and epidemiological studies strongly indicate metals have a participatory role in neurodegenerative diseases, but whether these roles are pathogenic and not simply subsidiary mechanisms is currently unclear. For Parkinson's disease (PD), iron content in the afflicted brain region, the substantia nigra pars compacta (SNpc), has been consistently reported elevated whereas copper levels have been decreased. Both metals exhibit deleterious functions that occur during the late stages of PD, but mutations involving the regulation of these two metals have not been associated with PD. The pathogenesis of PD is undoubtedly multifaceted and may consist of various etiological factors participating in slow disease ascension. However, the extent to which certain factors may contribute to PD is unclear. To study whether iron and/or copper may facilitate a parkinsonian state, a computational model of neuronal metal regulation was initially developed and then the system was perturbed, corresponding to proposed etiological pathways for PD from the literature, in order to determine which iron- and copper-based pathogeneses would elicit a parkinsonian system. We report that a defective glutathione system and/or inhibited cellular iron efflux have the neurotoxic capacities to initiate a system characteristic of PD; furthermore, these capacities are greatly enhanced with mutated α-synuclein proteins.

1. Introduction

The etiology of Parkinson's disease (PD) has remained elusive despite comprehensive evidence correlating the disease with numerous environment toxins and gene mutations. From a genetic perspective, there are heterogeneous genotypes for familial PD that include eleven identified gene mutations encoding numerous mitochondrial proteins (PARK2, DJ-1, PINK1), the ubiquitin-proteasome system (UCHL1), the α-synuclein protein (SNCA) and other unidentified proteins (Farrer, 2006; Schapira, 2008). Parkinsonism-inducing drugs such as 6-hydroxyldopamine (6-OH-DOPA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat, rotenone, and epoxomicin have implicated environmental toxins in sporadic PD etiology and are valuable in the study of acute parkinsonian neurodegeneration (Melrose et al., 2006). These toxins initiate similar parkinsonisms through disparate mechanisms: 6-OH-DOPA generates cytosolic reactive oxygen species (ROS) and cytotoxic quinones; MPTP, paraquat, and rotenone are mitochondrial complex I inhibitors; and epoxomicin is a ubiquitin-proteasom pathway (UPP) inhibitor (McNaught et al., 2004; Bové et al., 2005; Melrose et al., 2006). The evidence for endogenous causes of parkinsonism is supported by epidemiological studies that correlate idiopathic PD with toxic workplace exposures, including organic solvents, industrial chemicals, pesticides, herbicides, and the trace metals iron, copper, lead, manganese, and aluminum (Rybicki et al., 1993; Gorell et al., 1997; Johnson et al., 1999; Olanow and Tatton, 1999; Powers et al., 2003).

This evidence thus supports an etiologic mechanism for PD in which a heterogeneous variety of discrete predispositions and exogenous toxins may lead to the clinical phenotype of PD (Warner and Schapira, 2003). This idea is substantiated by the enigmatic and deleterious role of α-synuclein accumulations found in both familial and idiopathic PD. Although the function of α-synuclein is undetermined (for a review see: Kahle et al., 2002), the ubiquity of fibrillar α-synuclein amyloids, called protofibrils, in both parkinsonian neurons and SNpc Lewy bodies (LBs) has drawn considerable attention to this protein in PD pathology (Spillantini et al., 1998). These accumulations are inducible through numerous pathways that are both secondary and causative in nature, but exactly which pathologic mechanism(s) is responsible remains unclear.

Current animal models have proven valuable in pathological studies of PD. Both human wild-type (hWT) and mutated α-synuclein models have been developed through transgenic mice (Kahle et al., 2000; Masliah et al., 2000; van der Putten et al., 2000; Matsuoka et al., 2001; Rathke-Hartlieb et al., 2001; Giasson et al., 2002; Lee et al., 2002b; Richfield et al., 2002) and viral vector (VV)-mediated gene expression in rats (Kirik et al., 2002; Klein et al., 2002; Lo Bianco et al., 2002; Mochizuki et al., 2006) to study the

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clinicopathological characteristics of α-synuclein (for reviews, see: Fernagut and Chesselet, 2004; Fleming et al., 2005; Melrose et al., 2006; Chesselet, 2008). These models are capable of recapitulating the putative role of α-synuclein neurotoxicity. However, reproducing the multifaceted accession of neurodegeneration has fundamental limitations in murine animals.

Transgenic overexpression of hWT and mutant α-synuclein is often too widespread and therefore incapable of reproducing the selective nigrostriatal degeneration observed in PD (Dauer and Przedborski, 2003; Kirik and Björklund, 2003; Chesselet, 2008). A proportionately slow progression is characteristic of PD, but according to Melrose et al. (2006) the most marked transgenic phenotypes utilize promoters that generate extensive α-synuclein expression to regions other than the striatum. Transgenic mice for doubly mutated (Thiruchelvam et al., 2004) and truncated (Tofaris et al., 2006; Wakamatsu et al., 2008) α-synuclein are capable of inducing selective nigral neuron loss, but transgenic, singly mutated α-synuclein has failed to yield this specific spatial pathology (Fernagut and Chesselet, 2004). Viral gene delivery for hWT α-synuclein overexpression induces highly selective nigrostriatal neurodegeneration (Kirik et al., 2002), and similar studies involving VV-mediated A53T and A30P overexpression have reported striatal dopamine (DA) and dopaminergic cell loss with α-synuclein aggregations (Klein et al., 2002; Lo Bianco et al., 2002; Yamada et al., 2004; Lauwers et al., 2007). However, the acute α-synuclein overexpression is uncharacteristic of PD and the spatial specificity does not accurately represent the early stages of pathogenesis (Chesselet, 2008). Thus, although these models have intrinsic advantages and practicality, they evidently lack the versatility required to accurately study various predispositions implicated in PD susceptibility.

To address the etiological equivocalities of both sporadic and familial PD, we applied novel approaches in computational biology by utilizing empirical data and observations to develop a theoretical biochemical system. This interdependent, computational model of a SNpc neuron was first constructed to exhibit common tendencies in neuronal homeostasis (Section 2.2). After developing a working system, some known biochemical interactions of PD were introduced to comparatively reproduce the stages of neurodegeneration (Section 2.3). These canonical conventions of *in silico* modeling are becoming widely popular for system characterization, especially in the study of complex neurodegenerative diseases for their inherent application of elucidating and proposing pathogenic mechanisms for prospective, non-computational investigations (Raichur et al., 2006; Kaushik et al., 2007; Vali et al., 2007; Lecca, 2008).

We recently reported our first stage of model development, which addressed fundamental components in PD pathology: α-synuclein aggregation, dopamine metabolism, lysosomal degradation, and the ubiquitin-proteasome system (Sass et al., 2009). In an effort to generate a more refined and comprehensive model of PD, the focus of this study resolved to copper, iron, and dopamine metabolism in SNpc neurons. The objective was to elucidate proposed pathogenic and etiologic factors in PD by qualitatively determining which predispositions in these processes would, or would not, elicit a parkinsonian system (as defined in Section 2.3).

1.1. Components investigated

Iron and copper metabolisms were the principal systems computationally characterized for analysis, with subsidiary metabolic processes incorporated as necessary. As opposed to α-synuclein, it is currently unclear whether metabolic defects for these metals have the capacity to be an etiological factor(s); the peculiar behaviors of both iron and copper have garnered particular interest in neurodegenerative studies, so their pathological implications are still being elucidated (Gerlach et al., 1994; Peery et al., 2002; Zecca et al., 2004a,b; Gaggelli et al., 2006; Bharucha et al., 2008; Arreguin et al., 2009). This section will summarize the current status of PD research involving these metals and the postulated implications they have for pathology.

1.1.1. Iron

To inhibit the deleterious effects of iron during normal processes, cellular iron flux is tightly regulated throughout the body, from duodenal uptake to intravascular transport and intracellular storage (for a review see Goswami et al., 2002; Hentze et al., 2004; Dunn et al., 2006). The extrapyramidal region typically expresses relatively heightened levels of iron (Koeppen, 1995), which naturally increase with age regardless of a diseased state (Zecca et al., 2004a,b). During mild to severe PD, total SNpc iron levels increase selectively by 35% or greater depending directly on the disease severity (Sofic et al., 1988; Dexter et al., 1989; Riederer et al., 1989; Gorell et al., 1995; Berg and Youdim, 2006). Numerous mechanisms have been suggested for these increases, including mishandling caused by the oxidative stress in neurodegeneration (Jenner and Olanow, 1998; Zecca et al., 2004a,b; Barnham and Bush, 2008), genetic mutations in iron handling mechanisms (Borie et al., 2002; Hochstrasser et al., 2004, 2005; Berg et al., 2006), or altered vascularization associated with age and/or neurodegeneration (Faucheux et al., 1999).

Cytosolic iron, termed labile iron, is highly capable of producing hydroxyl radicals (•OH) via Fenton/Haber–Weiss chemistry as seen below (for a review see Kruszewski, 2003):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$

$$Fe^{3+} + {}^{\bullet}O_2{}^- \rightarrow Fe^{2+} + O_2$$

The role of iron as an •OH catalyst is evidenced in severe PD by the massive increases in Fe(III) and the Fe(II):Fe(III) ratio change of 3:1 to 1:1 in the SNpc (Sofic et al., 1988). This interconversion of iron redox states is fundamental in parkinsonian oxidative stress by inducing the oxidation of lipids, proteins, lipoproteins, DNAs, carbohydrates, and other intracellular structures (Kruszewski, 2003). Of these structures, parkinsonian ferritin proteins exhibits significantly increased iron loads (Griffiths et al., 1999) but diminished concentrations (Dexter et al., 1990, 1991), suggesting that the synthesis and/or structural integrity of ferritin is compromised.

Furthermore, in SNpc dopaminergic neurons, iron is concomitantly sequestered with DA in synaptic vesicles (Ortega et al., 2007), suggesting a direct relationship between α-synuclein accumulation, cytosolic iron and lipid peroxidation. Labile iron can induce α-synuclein misfolding and aggregation, thus presenting a tentatively deleterious cycle (Uversky et al., 2001; Wolozin and Golts, 2002; Cole et al., 2005); this is supported by *in vitro* evidence (Ostrerova-Golts et al., 2000) and by the iron deposits commonly found on LBs (Zecca et al., 2004a,b). Iron infusion into the SNpc of rodents induces a progressive, concentration-dependent degeneration of SNpc neurons, loss of striatal DA and motor impairment (Sengstock et al., 1993, 1994). Conversely, models treated with MPTP (Temlett et al., 1994) and 6-hydroxydopamine (6-OHDA) lesions (Oestreicher et al., 1994) exhibit increased SNpc iron, confirming the abilities of iron to either initiate neurodegeneration or accumulate secondarily in a parkinsonian system.

1.1.2. Copper

SN copper levels generally decrease with age and correlate with a reduction in copper-dependent enzymatic processes (Religa et al., 2006); and this trend is consistent with additional copper decreases in parkinsonian SN (Dexter et al., 1989, 1991; Barnham and Bush, 2008). The cause of this decrease is uncertain but can be speculated: the copper enzyme tyrosine hydoxylase (TH) catalyzes DA