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Inducible Alterations of Glutathione Levels in Adult Dopaminergic Midbrain Neurons Result in Nigrostriatal Degeneration

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Parkinson's disease is a neurodegenerative disorder characterized by the preferential loss of midbrain dopaminergic neurons in the substantia nigra (SN). One of the earliest detectable biochemical alterations that occurs in the Parkinsonian brain is a marked reduction in SN levels of total glutathione (glutathione plus glutathione disulfide), occurring before losses in mitochondrial complex I (CI) activity, striatal dopamine levels, or midbrain dopaminergic neurodegeneration associated with the disease. Previous *in vitro* data from our laboratory has suggested that prolonged depletion of dopaminergic glutathione results in selective impairment of mitochondrial complex I activity through a reversible thiol oxidation event. To address the effects of depletion in dopaminergic glutathione levels *in vivo* on the nigrostriatal system, we created genetically engineered transgenic mouse lines in which expression of γ-glutamyl cysteine ligase, the rate-limiting enzyme in *de novo* glutathione synthesis, can be inducibly downregulated in catecholaminergic neurons, including those of the SN. A novel method for isolation of purified dopaminergic striatal synaptosomes was used to study the impact of dopaminergic glutathione depletion on mitochondrial events demonstrated previously to occur *in vitro* as a consequence of this alteration. Dopaminergic glutathione depletion was found to result in a selective reversible thiol-oxidation-dependent mitochondrial complex I inhibition, followed by an age-related nigrostriatal neurodegeneration. This suggests that depletion in glutathione within dopaminergic SN neurons has a direct impact on mitochondrial complex I activity via increased nitric oxide-related thiol oxidation and age-related dopaminergic SN cell loss.

Key words: **glutathione; Parkinson's disease; mitochondrial complex I; dopaminergic neuron; *S*-nitrosation; substantia nigra**

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the preferential loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) as well as other brain regions. The pathogenesis of PD is not fully understood, but several lines of evidence suggest it may involve both increased oxidative stress and mitochondrial dysfunction. One of the earliest detectable biochemical alterations reported in parkinsonian SN is a marked reduction in total glutathione [glutathione (GSH) plus glutathione disulfide (GSSG)] levels, occurring before selective decreases in mitochondrial complex I activity, striatal dopamine loss, and dopaminergic neurodegeneration (Sian et al., 1994). Although glutathione is not the only antioxidant reported to be altered in PD, the magnitude of depletion parallels severity of the disease. Glutathione levels are not reduced in other areas in the parkinsonian brain or in other diseases affecting the SN, such as multiple system atrophy or progressive supranuclear palsy (Perry and Yong, 1986).

Glutathione plays an important role in brain by removing oxidants formed during metabolic processes, such as oxygen utilization by the mitochondria. It is one of the most abundant soluble antioxidant molecules present in brain, comprising cysteine, glycine, and glutamate. GSH is formed by a two-step reaction involving enzymes γ-glutamyl cysteine ligase (GCL) and glutathione synthetase. Brain GSH appears to arise primarily through *de novo* synthesis from its constituent amino acids via these enzymes (Meister, 1988). GCL is the rate-limiting enzyme in this process and composed of a heavy catalytic subunit (GCLC, 73 kDa) and a light modulatory subunit (GCLM, 27.7 kDa). The modulatory subunit increases the affinity for the substrate glutamate and decreases sensitivity to feedback inhibition by glutathione (Huang et al., 1993). Previous *in vitro* studies from our laboratory demonstrated that acute reduction in both cellular and mitochondrial glutathione levels in dopaminergic PC12 cells resulted in mitochondrial dysfunction (Jha et al., 2000). Recent studies from our laboratory and others demonstrated that both acute and chronic *in vitro* dopaminergic glutathione depletion results in reversible, selective inhibition of complex I activity via nitric oxide (NO)-mediated thiol oxidation (Hsu et al., 2005; Chinta et al., 2006; Burwell et al., 2006). Recently, GCLC knock-

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