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MPTP and DSP-4 susceptibility of substantia nigra and locus coeruleus catecholaminergic neurons in mice is independent of parkin activity

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Mutations in the parkin gene cause autosomal recessive familial Parkinson's disease (PD). Parkin-deficient mouse models fail to recapitulate nigrostriatal dopaminergic neurodegeneration as seen in PD, but produce deficits in dopaminergic neurotransmission and noradrenergic-dependent behavior. Since sporadic PD is thought to be caused by a combination of genetic susceptibilities and environmental factors, we hypothesized that neurotoxic insults from catecholaminergic toxins would render parkin knockout mice more vulnerable to neurodegeneration. Accordingly, we investigated the susceptibility of catecholaminergic neurons in parkin knockout mice to the potent dopaminergic and noradrenergic neurotoxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) respectively. We report that nigrostriatal dopaminergic neurons in parkin knockout mice do not show increased susceptibility to the parkinsonian neurotoxin, MPTP, in acute, subacute and chronic dose regimens of the neurotoxin. Additionally, parkin knockout mice do not show increased vulnerability to the noradrenergic neurotoxin, DSP-4, regarding levels of norepinephrine in cortex, brain stem and spinal cord. These findings suggest that absence of parkin in mice does not increase susceptibility to the loss of catecholaminergic neurons upon exposure to both dopaminergic and noradrenergic neurotoxins.

Keywords: Parkinson's disease; Parkin; Alpha-synuclein; MPTP; DSP-4; Substantia nigra; Locus coeruleus; Dopamine; Norepinephrine

Introduction

Parkinson's disease (PD) is a debilitating disorder marked by a prominent and progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with the presence of intraneuronal protein inclusions called Lewy bodies (Forno, 1987; Lang and Lozano, 1998). Clinical manifestations of PD include motor impairments involving resting tremor, bradykinesia, postural instability and rigidity. In addition to SNpc, other catecholaminergic neurons, including the locus coeruleus, as well as olfactory nuclei, sympathetic ganglia and also a substantial number of neurons in the pedunculopontine nucleus and median raphe degenerate in PD (Halliday et al., 1990; Braak et al., 2003; Micieli et al., 2003; Zarow et al., 2003). The molecular mechanisms leading to the degeneration of nigral dopaminergic neurons in PD are unclear; however a combination of genetic susceptibilities and environmental factors appears to play a critical role (Greenamyre and Hastings, 2004). Although the majority of PD cases are sporadic, identification of genes linked to rare forms of familial PD has yielded significant insights into pathogenesis of PD.

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Inherited mutations in *PARK2*, the gene encoding parkin, an E3 ubiquitin protein ligase, cause selective degeneration of catecholaminergic neurons in the substantia nigra and locus coeruleus of the brainstem, resulting in early-onset parkinsonism (Kitada et al., 1998; Zhang et al., 2000). A number of putative substrates for parkin have been identified, and the accumulation of one or several of these substrates may account for the neurodegeneration in patients with loss of function-parkin mutations by impairment of ubiquitin–proteasomal degradation (von Coelln et al., 2004a; Ko et al., 2005). Parkin has been suggested to function as a multipurpose neuroprotective agent against a variety of toxic insults and is thought to be critical for survival of dopaminergic neurons (Feany and Pallanck, 2003). These include alpha-synuclein toxicity (Petrucelli et al., 2002; Lo Bianco et al., 2004), proteasomal dysfunction (Tsai et al., 2003), Pael-R (Yang et al., 2003), accumulation of the authentic parkin substrate p38/JTV-1 (Ko et al., 2005), kainate-induced excitotoxicity (Staropoli et al., 2003), dopamine toxicity (Jiang et al., 2004), and toxins that impair mitochondrial function (Darios et al., 2003). Additionally, impairment of the catalytic activity of parkin either due to familial PD-linked mutations or due to nitrosative, oxidative or dopaminergic stress contributes to parkin dysfunction (Chung et al., 2004; Kalia et al., 2004; LaVoie et al., 2005; Sriram et al., 2005; Wang et al., 2005a, b). Moreover, parkin may be a critical factor in the normal functioning of key cellular processes that involve maintenance of mitochondrial function (Greene et al., 2003, Palacino et al., 2004), expression of monoamine oxidases (Jiang et al., 2006), stability of microtubules (Yang et al., 2005), and signaling of cell survival pathways (Cha et al., 2005). The dysfunction of these processes could potentially impair dopamine neuron survival. Understanding how lack of parkin activity or its dysfunction is linked to the degeneration of catecholaminergic nigral and locus coeruleus neurons in individuals with parkin-associated familial PD should provide insights into the pathogenic mechanisms causing the much more common sporadic form of PD.

Epidemiological studies indicate that exposure to pesticides, rural living, farming, and drinking well water are associated with an increased risk of developing PD (Gorell et al., 1998). There is increasing evidence, which suggests that these environmental factors cause oxidative stress by affecting mitochondrial functions and key cell survival pathways that play important roles in the pathogenesis of PD. These environmental agents include toxins that interfere with normal functioning of mitochondria, increase oxidative stress, and impair the ubiquitin–proteasome system in dopaminergic neurons, leading to their degeneration (Dawson and Dawson, 2003; Warner and Schapira, 2003). The lack of cardinal pathological features relevant to PD in mouse models of parkin deficiency prompted us to investigate the existence of a potential link between environmental factors and how mutations in parkin cause parkinsonism, by using the previously described mouse model with targeted disruption of the mouse parkin gene (Von Coelln et al., 2004b). Our parkin knockout mice show partial loss of catecholaminergic locus coeruleus neurons associated with reduced levels of norepinephrine in discrete brain regions and a marked reduction of the norepinephrine-dependent acoustic startle response; however, the nigrostriatal dopaminergic system appears to be intact (Von Coelln et al., 2004b).

In the present study we investigated how neurotoxins selective to central catecholaminergic neurons affect survival of these PD-associated neuronal populations in a mouse model of parkin deficiency. The parkin knockout mice were treated either with the potent parkinsonian neurotoxin, MPTP (Dauer and Przedborski, 2003), that causes selective degeneration of nigral dopaminergic neurons, or with the central noradrenergic neurotoxin, DSP-4, that induces selective degeneration of noradrenergic axon terminals originating in the locus coeruleus (Jonsson et al., 1981; Fritschy and Grzanna, 1989). We show that lack of parkin fails to increase the vulnerability of SNpc dopaminergic neurons to MPTP toxicity in acute, subacute and chronic dose regimens both in young and old animals. Furthermore, compared to age-matched wild-type littermates, parkin-deficient mice are not more susceptible to the DSP-4-induced degeneration of noradrenergic axon terminals, as determined by measuring norepinephrine levels in discrete brain regions. Taken together, we provide evidence that absence of parkin does not render murine central catecholaminergic neurons more susceptible to neurotoxins commonly used for animal models of neurodegenerative disease. These results therefore argue against a critical role of parkin in the neuronal defense mechanism against exogenous, and possibly also environmental, toxic factors.

Materials and methods

Animals

All mice were housed and treated in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Mice were housed in a pathogen-free facility, 4–5 animals per cage in a temperature-controlled room with a 12-h light/dark cycle and access to food and water ad libitum. All procedures were approved by and conformed to the guidelines of the Institutional Animal Care Committee of Johns Hopkins University. We generated parkin knockout mice and age-matched littermate controls by crossing parkin heterozygous mice as described previously (Von Coelln et al., 2004b). The wild-type and parkin knockout mice used in the present study were backcrossed to C57Bl6 mice for (N6) six generations. Age and sex matched littermate cohorts were used for all the experiments to account for potential strain differences.

PCR and Western blot analysis

Genotyping was performed by PCR analysis of genomic DNA, using the following primers: exon 7 sense, AATGGATGAGTTCAAGGTTGCACAG; exon 7 antisense, AACTCCAGAGCTAGGATAGGGCATA. The amplification products were separated on a 1% agarose gel for visualization (Von Coelln et al., 2004b). To assess parkin expression, wild-type, heterozygous and parkin null mice were decapitated, and the brains were dissected. Lysis was performed in buffer A (10 mM Tris–HCl, pH 7.4/150 mM NaCl/5 mM EDTA/0.5% Nonidet P-40/10 mM Na–glycerophosphate/Phosphatase Inhibitor Mixture 1 and 2 (Sigma, St. Louis, MO)/Complete Protease Inhibitor Mixture (Roche, Indianapolis, IN)), by using a Diax 900 homogenizer (Heidolph, Cinnaminson, NJ). After homogenization, samples were rotated at 4 °C for 30 min for complete lysis, and then centrifuged (100,000×*g*, 4 °C, 20 min). The pellet was discarded. Proteins were separated by SDS/PAGE and electroblotted onto a poly (vinylidene difluoride) membrane (Bio-Rad, Hercules, CA). Immunolabeling was carried out by using an anti-parkin primary antibody [PRK8, mouse monoclonal] (Pawlyk et al., 2003; Von Coelln et al., 2004b), a horseradish peroxidase-conjugated secondary antibody (Amersham Pharmacia Biosciences, Piscataway, NJ), and ECL solutions (Amersham