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DIFFERENTIAL CONTRIBUTION OF LIPOXYGENASE ISOZYMES TO NIGROSTRIATAL VULNERABILITY

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Abstract—The 5- and 12/15-lipoxygenase (LOX) isozymes have been implicated to contribute to disease development in CNS disorders such as Alzheimer's disease. These LOX isozymes are distinct in function, with differential effects on neuroinflammation, and the impact of the distinct isozymes in the pathogenesis of Parkinson's disease has not as yet been evaluated. To determine whether the isozymes contribute differently to nigrostriatal vulnerability, the effects of 5- and 12/15-LOX deficiency on dopaminergic tone under naïve and toxicant-challenged conditions were tested. In naïve mice deficient in 5-LOX expression, a modest but significant reduction (18.0% reduction vs. wildtype (WT)) in striatal dopamine (DA) was detected (n = 6–8 per genotype). A concomitant decline in striatal tyrosine hydroxylase (TH) enzyme was also revealed in null 5-LOX vs. WT mice (26.2%); however, no changes in levels of DA or TH immunoreactivity were observed in null 12/15-LOX vs. WT mice. When challenged with the selective dopaminergic toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), WT mice showed a marked reduction in DA (31.9%) and robust astrocytic and microglial activation as compared to saline-treated animals. In contrast, null 5-LOX littermates demonstrated no significant striatal DA depletion or astrogliosis (as noted by Western blot analyses for glial acidic fibrillary protein (GFAP) immunoreactivity). In naïve null 12/15-LOX mice, no significant change in striatal DA values was observed compared to WT, and following MPTP treatment, the transgenics revealed striatal DA reduction similar to the challenged WT mice. Taken together, these data provide the first evidence that: (i) LOX isozymes are involved in the maintenance of normal dopaminergic function in the striatum and (ii) the 5- and 12/15-LOX isozymes contribute differentially to striatal vulnerability in response to neurotoxicant challenge.

Key words: Parkinson's disease, nigrostriatal, dopamine, lipoxygenase, MPTP, inflammation.

INTRODUCTION

Parkinson's disease (PD) is a progressive CNS disorder characterized behaviorally by motor impairment and neurochemically by a loss of nigrostriatal dopaminergic tone. Fundamental processes thought to underlie ongoing degeneration in PD include oxidative damage, mitochondrial dysfunction, protein clearance abnormalities and protein misfolding (Imai and Lu, 2011; Plowey and Chu, 2011; Breydo et al., 2012; McCoy and Cookson, 2012). Inflammation has been implicated to contribute to PD pathogenesis as revealed by epidemiological and pathological studies as well as experimental modeling but whether these are primary or secondary processes is not well understood (Hunot and Hirsch, 2003; Tansey et al., 2008; Ouchi et al., 2009; Ton et al., 2012). Glial involvement (i.e. astrogliosis and microglial activation) in cell death and dysfunction have been demonstrated to play a key role (Ton et al., 2006, 2012; Tansey et al., 2008; Tansey and Goldberg, 2010), and eicosanoids, the collective name for products of arachidonic acid (AA) metabolism, have been widely studied in mediating activation at a cellular level (Minghetti, 2004; Farooqui and Horrocks, 2006; Farooqui et al., 2007; Adibhatla and Hatcher, 2008). The lipoxygenases (LOX) are a family of distinct isozymes that catalyze oxidation of AA and consequently contribute to toxicity through the generation of free reactive oxygen species (ROS), toxic lipid hydroperoxides and potent cytokines (Serhan and Samuelsson, 1988; Zaleska and Wilson, 1989; Simonian and Coyle, 1996; Manev, 2000; Sugaya et al., 2000; Mytilineou et al., 2002; Parkinson, 2006; Serhan, 2006; Yang et al., 2010). The LOX enzymes have been shown to play a central role in pathogenesis in a variety of disease states including diabetes, asthma, cancer, heart disease, and most recently, neurodegenerative conditions (Serhan and Samuelsson, 1988; Parkinson, 2006; Serhan, 2006; Cheng et al., 2008; van Leyen et al., 2008; Weaver et al., 2012; Yeung et al., 2012) including Alzheimer's disease (AD) (Pratico et al., 2004; Manev and Manev, 2006; Listi et al., 2010; Yang et al.,

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Abbreviations: AA, arachidonic acid; AD, Alzheimer's disease; ANOVA, analysis of variance; DA, dopamine; DAB, 3,3′-diaminobenzidine; DOPAC, dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; GFAP, glial acidic fibrillary protein; HETE, hydroxyeicosatetraenoic acid; HVA, homovanillic acid; LOX, lipoxygenase(s); LTB_4, leukotriene B_4; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PPARγ, peroxisome proliferator activator receptor gamma; ROS, reactive oxygen species; TH, tyrosine hydroxylase; WT, wildtype.

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2010; Chu and Pratico, 2011; Manev et al., 2011; Puccio et al., 2011) and Creutzfeldt–Jakob disease (Stewart et al., 2001; Phillis et al., 2006). Despite that LOX isozymes are well established in lipid-mediated cellular responses, understanding of their role(s) in the nigrostriatal pathway represents a gap in knowledge.

All LOX isozymes are expressed in the brain (Bendani et al., 1995; Manev et al., 2000a, 2001; Qu et al., 2000; Uz et al., 2001a,b) including the ventral midbrain which contains the nigrostriatal neuronal cell bodies affected in PD (Mytilineou et al., 1999). The expression of LOX is enhanced in the brain with aging and in models of neurodegeneration and inflammation (Uz et al., 1998; Qu et al., 2000; Manev et al., 2000b; Firuzi et al., 2008; Basselin et al., 2010). Recent studies related to CNS disease have focused primarily on the 5- and 12/15-LOX isozymes (Chu and Pratico, 2011; Hashimoto, 2011; Manev et al., 2011; Puccio et al., 2011; Chu et al., 2012a,b). The LOX isozymes catalyze stereospecific modification of AA at either carbon position 5, 12 or 15 or both 12 and 15, and the site of molecular oxygen insertion correspondingly defines the isozyme (i.e. 5-, 12-, 15- or a dual specificity 12/15-LOX, respectively). The reaction product of AA oxidation is hydroperoxyeicosatetraenoic acid (HPETE); this unstable compound is subsequently reduced by glutathione peroxidase to the more stable hydroxyeicosatetraenoic acid (HETE) (Brash, 1999). While 5-, 12-, 15- and 12/15-LOX can generate HETE forms, the distinct products have selective downstream effects. For example, 12-HETE, but not 5-HETE, antagonizes the activity of the peroxisome proliferator activator receptor gamma (PPARγ) to promote glial cell activation (Lopez-Parra et al., 2005; Limor et al., 2008). Interestingly, 5-HETE is also a substrate for the 5-LOX isozyme, catalyzing the formation of the epoxide, leukotriene A_4, which is subsequently converted to leukotriene B_4 (LTB_4). LTB_4 is a highly potent inflammatory factor and promotes enhanced expression of IL-12, IL-6 and TNFα (Phillis et al., 2006; Tassoni et al., 2008).

Although inhibition of 5- and 12/15-LOX has been shown to protect against the detrimental outcomes in disease models of stroke and AD (Klegeris and McGeer, 2002; Wang et al., 2006; Jin et al., 2008; Sobrado et al., 2009; Cui et al., 2010; Tu et al., 2010), the role of these isozymes in the nigrostriatal pathway has not been fully evaluated. The development and availability of mouse models with targeted gene deletions provide critical tools to better understand the role of LOX in degeneration related to PD. To determine if the 5- and 12/15-LOX isozymes selectively contribute to nigrostriatal vulnerability, the impact of 5- or 12/15-LOX deficiency on striatal dopaminergic tone and injury was tested in mouse models under naïve and toxicant-challenged conditions.

EXPERIMENTAL PROCEDURES

Animals

Transgenic mice deficient in 5-LOX (004155; sex-matched and aged 8 weeks) or 12/15-LOX (002778; males aged 8 weeks) and respective sex-matched strain controls were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed on a 12-h light/dark cycle and given free access to food and drinking water as well as environmental enrichment. All animal procedures and animal care methods were approved by the Institutional Animal Care and Usage Committees for SRI International.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposure. The dopaminergic neurotoxin MPTP (Sigma, St. Louis, MO) was dissolved in sterile saline. Mice received saline or 15 mg/kg MPTP, i.p., daily for 5 days. All animals were weighed daily during MPTP administration and monitored until 72 h after the last MPTP exposure. Animals were euthanized by cervical dislocation 7 days following the last MPTP injection.

Tissue processing

Midbrain was immersion-fixed in 4% paraformaldehyde overnight, cryoprotected in graded sucrose solutions and frozen at −80 °C. Coronal sections were collected on a Leica cryostat (Buffalo Grove, IL) at 40-μm thickness for histology. Striatal tissue was dissected from a forebrain slice (1–2 mm thick) at the level of the anterior commissure and frozen immediately on dry ice. Tissues were sonicated and centrifuged, and homogenate fractions collected for biochemical analyses by Western blot and neurochemical analyses of dopamine (DA) and metabolite levels.

Neurochemistry. Striatal samples were placed in 1-ml ice-cold 0.4 M perchloric acid, sonicated and centrifuged at 15,000*g* for 12 min. The supernatant was collected for assay of DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) by HPLC with electrochemical detection (Coulochem III detector; Dionex/Thermo Scientific) using a reverse phase C18 column (MD-150; Dionex/Thermo Scientific) (Kilpatrick et al., 1986). The pellet was dried and reconstituted in 0.5 N NaOH and briefly sonicated, and total protein determined using the Lowry method (Manning-Bog et al., 2007).

Immunoblotting. Proteins separated by the sample preparation were used for immunoblotting experiments. Following sonication in Tris–EDTA buffer with protease and phosphatase inhibitors (Sigma), samples were centrifuged at 1000*g* for 10 min, the supernatant aspirated, and the pellet reconstituted in sample buffer. The protein concentration was measured by Pierce BCA assay (Thermo Scientific, Rockford, IL) and supernatant proteins separated by SDS–PAGE (12% Tris–glycine, Invitrogen/Life Technologies, Carlsbad, CA) then transferred to nitrocellulose. Blots were blocked in 5% non-fat milk and incubated overnight at 4 °C with rabbit anti-tyrosine hydroxylase (TH; Pel-Freez Biologicals, Rogers, AK; and Chemicon/Millipore, Billerica, MA), rabbit anti-β-actin (Sigma), and mouse anti-glial acidic fibrillary protein (GFAP; Covance Inc., Princeton, NJ). Next, appropriate peroxidase-conjugated secondary antibodies were applied, and signal visualized following incubation with Pierce chemiluminescent substrate (Thermo Scientific). Blots were placed in plastic sleeves and exposed to CL-XPosure Film (Thermo Scientific), and immunoreactivity was quantified by Image J software.

Immunohistochemistry. Coronal tissue sections (40 μm) were obtained as described above and stored in cryoprotectant solution (0.01 M phosphate buffer, 30% sucrose, 30% ethylene glycol, pH 7.4) at −20 °C until further use. The standard protocol that was used to detect primary antibodies has been described previously (Manning-Bog et al., 2003). Incubation times varied depending on the primary and secondary antibodies that were utilized. Antibodies used were rabbit and sheep anti-TH (Pel-Freez and Chemicon/Millipore), rabbit anti-GFAP (Chemicon/Millipore), and rabbit anti-Iba1 (Biocare