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LOSS OF GLUTAMIC ACID DECARBOXYLASE (Gad67) IN STRIATAL NEURONS EXPRESSING THE Drdr1a DOPAMINE RECEPTOR PREVENTS L-DOPA-INDUCED DYSKINESIA IN 6-HYDROXYDOPAMINE-LESIONED MICE

K. ZHANG, C. CHAMMAS AND J.-J. SOGHOMONIAN *

Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118, USA

Abstract—The objective in this study was to test the hypothesis that the GABA-synthesizing enzyme, glutamic acid decarboxylase (Gad67), expressed in striatal neurons plays a key role in dyskinesia induced by L-DOPA (LID) in a rodent model of Parkinson's disease. In light of evidence that the dopamine Drd1a receptor is densely expressed in striatal direct pathway striatal neurons while the orphan G-protein-coupled receptor Gpr88 is densely expressed in striatal direct and indirect pathway striatal neurons, we used a cre-lox strategy to produce two lines of mice that were Gad1 (Gad1 is the gene encoding for Gad67)-deficient in neurons expressing the Drd1a or the Gpr88 receptor. Gad67 loss in Gpr88-expressing neurons mice did not result in gross motor abnormalities while mice with Gad67 loss in Drd1a-expressing neurons were impaired on the Rotarod and the pole test. Knockout and control littermate mice were unilaterally injected into the medial forebrain bundle with 6-hydroxydopamine (6-OHDA) in order to lesion dopamine neurons on one side of the brain. 6-OHDA-lesioned mice were then injected once daily for 10 days with L-DOPA. Mice with a Gad67 loss in Gpr88-expressing neurons and control littermates developed abnormal involuntary movements (AIM), a measure of dyskinesia. In contrast, mice with a Gad67 loss in Drd1a-expressing did not develop AIM. The results demonstrate that Gad67 in Drd1a-expressing neurons plays a key role in the development of LID and they support the hypothesis that altered GABAergic neurotransmission in the direct pathway is involved in dyskinesia.

Key words: GABA, 6-hydroxydopamine, levodopa, dyskinesia, striatum, striatonigral.

INTRODUCTION

Levodopa (L-DOPA), the metabolic precursor of dopamine, is an agent commonly used for the symptomatic treatment of Parkinson's disease. However, long-term exposure to L-DOPA in Parkinson's disease patients or in experimental models of Parkinson's disease can induce abnormal involuntary movements (AIM) known as L-DOPA-induced dyskinesia (LID). The mechanisms involved in LID are not fully understood but they are known to involve an abnormal activation of neuronal and biochemical pathways in the basal ganglia. In particular, there is evidence that the striatal direct projection to the substantia nigra plays a critical role in LID (Bateup et al., 2010; Murer and Moratalla, 2011; Valjent, 2012; Heiman et al., 2014). Dopamine Drd1a receptors, which are highly enriched in striatonigral neurons (Le Moine and Bloch, 1995; Surmeier et al., 1996), play a central role in LID. Pharmacological blockade of D1 receptors opposes the dyskinesiogenic effects of L-DOPA in neurotoxin-generated experimental models of Parkinson's disease (Monville et al., 2005; Taylor et al., 2005; Westin et al., 2007; Murer and Moratalla, 2011; Mela et al., 2012). Consistent with these pharmacological data, genetic inactivation of dopamine D1 receptors prevents LID (Darmopil et al., 2009). On the other hand, there is evidence that striatal indirect pathway projection neurons (striatopallidal pathway), which preferentially express the dopamine D2 and adenosine A2A receptors, are also involved in LID (Svenningsson et al., 1997; Fredduzzi et al., 2002; Jenner, 2003) but the importance of this pathway to LID and the mechanisms that link it to LID are unclear.

Striatal projection neurons use the neurotransmitter GABA and it is likely that GABA released by striatal projection neurons plays a role in LID. In support of this possibility, studies have shown that the chronic administration of L-DOPA to dopamine-depleted rodents or monkeys increases gene expression of the GABA-synthesizing enzyme glutamic acid decarboxylase (Gad) in striatal neurons (Soghomonian et al., 1996; Cenci et al., 1998) and increases GABA release in the substantia nigra (Yamamoto et al., 2006; Mela et al., 2007, 2012). In addition, the co-administration of agents that decrease the severity of LID also decreases Gad gene expression in the striatum (Yamamoto and Soghomonian, 2009) or GABA release in the substantia nigra (Mela et al., 2007,

*Corresponding author. Address: Boston University School of Medicine, 72E. Concord Street, Room L-1001, Boston, MA 02118, USA.
E-mail address: jjsogho@bu.edu (J.-J. Soghomonian).
Abbreviations: 6-OHDA, 6-hydroxydopamine; AIM, abnormal involuntary movements; ANOVA, Analysis of variance; Gad, glutamic acid decarboxylase; L-DOPA, levodopa; LID, L-DOPA-induced dyskinesia.

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2012; Bido et al., 2011). The effects of L-DOPA on Gad gene expression are particularly prominent on the Gad67 isoform and are predominantly detected in direct pathway neurons (Carta et al., 2003; Nielsen and Soghomonian, 2004), a finding consistent with the hypothesis that GABA in the direct pathway plays a role in LID. However, the infusion of GABA in the substantia nigra failed to evoke AIM in a rodent neurotoxin model of Parkinson's disease (Buck et al., 2010). Therefore the contribution to LID of GABA produced in striatal neurons remains controversial. The objective in this study was to further test the hypothesis that Gad67 is involved in LID. In order to test this hypothesis, we bred mice with a floxed *Gad1* gene (gene encoding for the Gad67 isoform of Gad) with mice expressing Cre-recombinase under the promotor activity of the dopamine *Drd1a* receptor, which is densely expressed in striatal direct pathway neurons (Le Moine and Bloch, 1995; Surmeier et al., 1996), or the orphan G-protein-coupled receptor Gpr88, which is primarily and densely expressed in both striatal direct and indirect pathway neurons (Massart et al., 2009; Van Waes et al., 2011; Hisatsune et al., 2013).

EXPERIMENTAL PROCEDURES

Animals

Mice were housed in a facility under a 12:12-h light:dark cycle. Food and water were available *ad libitum*. Experiments were carried out on adult mice between 2 and 6 months of age that were extensively backcrossed to C57BL/6 mice. All animal protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Boston University School of Medicine and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Generation of mutant mice

The breeding strategy in this study was chosen to avoid possible recombination at the *Gad1* locus by action of Cre recombinase during germ cell development. $Drd1a^{Cre}$ mice were BAC-engineered to express Cre-recombinase under the Drd1a promotor and where obtained from the MMRC (B6.FVB(Cg)-Tg(Drd1a-cre)EY262Gsat/Mmucd). $Gpr88^{CreGFP}$, $Gad1^{lox/lox}$ and $Gad1^{\Delta/+}$ mouse lines were a gift from Dr. Richard Palmiter (University of Washington). $Gpr88^{CreGFP}$ heterozygous mice have a Cre-GFP construct targeted to the first coding exon of the Gpr88 locus and thus express Cre-recombinase under activity of the *Gpr88* promotor (Quintana et al., 2012). $Gad1^{lox/lox}$ mice have exon 2 of the *Gad1* gene flanked by loxP sites (Chattopadhyaya et al., 2007). $Gad1^{\Delta/+}$ mice are heterozygous with a deleted *Gad1* allele (Heusner et al., 2008). In our experiments, heterozygous $Gad1^{\Delta/+}$ mice were bred with heterozygous $Gpr88^{CreGFP}$ mice to produce $Gpr88^{CreGFP}::Gad1^{\Delta/+}$ mice. $Gpr88^{CreGFP}::Gad1^{\Delta/+}$ mice were then bred with $Gad1^{lox/lox}$ mice. Of the four possible offspring genotypes, we used the $Gpr88^{CreGFP}::Gad1^{\Delta/lox}$ as a *Gad1* knockout since the action of Cre recombinase on the *Gad1* gene should lead to deletion of exon 2, and thus causes the mRNA to be out of frame for translation. The $Gpr88^{+/+}::Gad1^{\Delta/lox}$ genotype was used as control. Genotyping was carried out on genomic DNA isolated from tail tissue using standard techniques. The following primers were used for the *Gad1* gene: forward primer (5′-GCT ACT GTG CTT GCG CCC CAG TC-3′) and reverse primer (5′-GTA ACC TCG CGT CCC TGG CAG CC-3′) and for the *Gpr88* gene: forward primer (5′-TGG AGG AAC GAG GAG TTC CGC-3′) and reverse primer (5′-AGA AGG AGG CAG TGC GGC AGG-3′). In order to generate the other knockout $Drd1a^{Cre}::Gad1^{\Delta/lox}$ mice, female $Drd1a^{Cre}::Gad1^{+/lox}$ mice were crossed with male $Gad1^{\Delta/lox}$ mice. For the controls, we used $Drd1a^{+/+}::Gad1^{\Delta/lox}$ mice. Genotyping for *Drd1a* mutant mice was carried out with forward primer (5′-GCT ATG GAG ATG CTC CTG ATG GAA-3′) and reverse primer (5′-CCC CAG AAA TGC CAG ATT ACG TAT-3′). Following DNA amplification, the PCR products were detected on 2% agarose gels.

6-Hydroxydopamine (6-OHDA) lesions

In order to induce a unilateral lesion of dopamine neurons, mice received 6-OHDA injections into the left medial forebrain bundle (MFB) (Francardo et al., 2011). Anesthesia was induced with 2–3% isoflurane in air, and maintained with 1% isoflurane in air. The mouse was placed in a stereotaxic frame for mouse. 6-OHDA (Sigma, St-Louis, USA) was dissolved at a concentration of 3.2 μg/μl in 0.1% ascorbic acid and saline prior to surgery. One microliter was injected into the MFB using a Neuros™ syringe (Hamilton Co., USA) at a rate of 0.5 μl/min. The needle was left in place for 5 min before and after injection. The following coordinates relative to bregma determined the area of injection in the MFB: AP = +1.25, L = −0.6, DV = −5.3. Mice received a subcutaneous injection of sterile saline solution (0.1 ml/10 g body weight) immediately following surgery to prevent dehydration and two times a day during the two week recovery period. All mice showed difficulty eating and were force-fed twice a day with a solution of (per 1000 ml) glucose 50 g, NaCl 1 g, Na acetate trihydrate 3.13 g, KCl 1.5 mg and MgCl hexahydrate 300 g. Following a two week recovery period, mice were provided food and water *ad libitum*. To avoid food competition, mice of similar genotype and body weight were paired in the same cage. To assess the efficacy of the 6-OHDA lesion, mice were observed for evidence of circling behavior ipsilateral to the lesion. Mice that did not display ipsilateral circling were removed from the experiment.

Rota-Rod and pole test

These two tests were used to assess the presence and extent of motor deficits induced by the Gad67-deficiency and by 6-OHDA (Iancu et al., 2005; Glajch et al., 2012; Smith et al., 2012). Motor coordination and balance were assessed using the Five Station Rota-Rod Stand Alone for Mouse (Med Associates Inc., St. Albans, VT, USA). The machine was set to a progressive accelerating speed