Neuroscience 310 (2015) 12–26

ALTERATIONS IN PRIMARY MOTOR CORTEX NEUROTRANSMISSION AND GENE EXPRESSION IN HEMI-PARKINSONIAN RATS WITH DRUG-INDUCED DYSKINESIA

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Abstract—Treatment of Parkinson's disease (PD) with dopamine replacement relieves symptoms of poverty of movement, but often causes drug-induced dyskinesias. Accumulating clinical and pre-clinical evidence suggests that the primary motor cortex (M1) is involved in the pathophysiology of PD and that modulating cortical activity may be a therapeutic target in PD and dyskinesia. However, surprisingly little is known about how M1 neurotransmitter tone or gene expression is altered in PD, dyskinesia or associated animal models. The present study utilized the rat unilateral 6-hydroxydopamine (6-OHDA) model of PD/dyskinesia to characterize structural and functional changes taking place in M1 monoamine innervation and gene expression. 6-OHDA caused dopamine pathology in M1, although the lesion was less severe than in the striatum. Rats with 6-OHDA lesions showed a PD motor impairment and developed dyskinesia when given L-DOPA or the D_1 receptor agonist, SKF81297. M1 expression of two immediate-early genes (c-Fos and ARC) was strongly enhanced by either L-DOPA or SKF81297. At the same time, expression of genes specifically involved in glutamate and GABA signaling were either modestly affected or unchanged by lesion and/or treatment. We conclude that M1 neurotransmission and signal transduction in the rat 6-OHDA model of PD/dyskinesia mirror features of human PD, supporting the utility of the model to study M1 dysfunction in PD and the elucidation of novel pathophysiological mechanisms and therapeutic targets.

Key words: Parkinson's disease, dyskinesia, L-DOPA, D_1 agonist, primary motor cortex, immediate-early gene.

INTRODUCTION

Parkinson's disease (PD) is principally caused by the loss of dopamine (DA) cells in the substantia nigra, leading to poverty of movement (Dauer and Przedborski, 2003; Jankovic, 2008). Treatment with L-DOPA relieves PD symptoms, but long-term use typically causes L-DOPA-induced dyskinesias (LID) that are in part due to supersensitization of DA D_1 receptors (Cenci et al., 2011; Feyder et al., 2011). An alternative strategy to treating PD has involved the use of primary motor cortex (M1) transcranial magnetic stimulation, which has shown promise in two meta-analyses (Fregni et al., 2005; Elahi et al., 2009).

Even though conventional anti-PD therapies modulate M1 activity and the region can be directly targeted for symptomatic relief, relatively little is known about how M1 monoamine transmission and gene expression are altered in human PD patients or in associated animal models (Lindenbach and Bishop, 2013). In the lone post-mortem study of M1 catecholamine fibers in PD patients, axons staining positively for tyrosine hydroxylase (TH; predominantly DA neurons: Hokfelt et al., 1977; Miner et al., 2006) were reduced by 24–74% compared to controls, depending on the cortical layer (Gaspar et al., 1991). In the popular 6-hydroxydopamine (6-OHDA) rat model of PD, reductions in M1 TH-positive fibers have been reported using optical density (Halje et al., 2012) or qualitative histology (Debeir et al., 2005), but there have been no attempts to rigorously quantify the extent of fiber loss. Changes in M1 monoamine tissue concentrations have not been assessed in humans or rat models of PD. Studies in parkinsonian primates have sometimes reported reductions in M1 DA, norepinephrine (NE) and serotonin (5-HT) levels, while other studies have found M1 monoamines to be equal to controls despite severe subcortical monoamine pathology (Pifl et al., 1991; Engeln et al., 2015). It is unclear how these changes in monoamine innervation effect cellular physiology in M1, although, at least in the prefrontal cortex, DA receptors modulate both glutamate and GABA currents (Lewis and O'Donnell, 2000; Seamans et al., 2001a,b). A similar pattern may be occurring in M1, as animal models of PD typically show abnormal firing patterns of M1 glutamatergic and GABAergic cells (Watts and Mandir,

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Abbreviations: 5-HIAA, 5-hydroxyindolacetic acid; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine; AIMs, Abnormal involuntary movements; ARC, activity-regulated cytoskeletal-associated protein; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; FAS, forepaw adjusting steps; GAD67, glutamic acid decarboxylase 67 kDa; HPLC, high-performance liquid chromatography; LID, L-DOPA-induced dyskinesia; M1, primary motor cortex; MFB, medial forebrain bundle; NE, norepinephrine; PCR, polymerase chain reaction; PD, Parkinson's disease; SKF, SKF81297; TH, tyrosine hydroxylase; Veh, vehicle; VGLUT1, vesicular glutamate transporter type I.

http://dx.doi.org/10.1016/j.neuroscience.2015.09.018
0306-4522/

1992; Parr-Brownlie and Hyland, 2005; Pasquereau and Turner, 2011; Brazhnik et al., 2012; Halje et al., 2012).

The influence of DA depletion and exogenous DA replacement on local M1 gene expression is unclear, although a key role for M1 DA is to facilitate motor learning, likely through promoting plasticity in M1 (Floel et al., 2005; Hosp and Luft, 2013). Under normal circumstances, motor learning in M1 is associated with DA-dependent induction of the immediate-early gene c-Fos: expression levels rise while learning a motor task and decline nearly to control levels with repeated performance of the task (Kleim et al., 1996; Hosp et al., 2011). Since LID is often viewed as a pathological form of motor learning that is coincident with *striatal* c-Fos induction, it is possible that *M1* c-Fos is involved in abnormal motor learning during LID (Calabresi et al., 2000, 2015; Mura et al., 2002). While multiple laboratories have reported that M1 c-Fos is induced by L-DOPA during dyskinesia (Ostock et al., 2011; Halje et al., 2012), these studies have been performed only in animals with multiple exposures to L-DOPA and the contribution of D_1 receptors to this effect is unclear. Whereas c-Fos is critical for affecting transcriptional activity, another immediate-early gene, activity-regulated cytoskeletal-associated protein (ARC), is important for promoting synaptic plasticity in part through AMPA receptor trafficking, and may identify unique aspects of cortical plasticity (Bramham et al., 2008; Korb and Finkbeiner, 2011; Perez-Cadahia et al., 2011). Indeed, ARC protein was recently shown to be preferentially enhanced by L-DOPA among rats that displayed significant LID as opposed to more stable L-DOPA responders (Bastide et al., 2014).

The goal of the present study was to characterize structural and functional changes occurring in M1 in a widely used rat model of PD/LID, in order to spur further research and highlight therapeutic approaches. First, 6-OHDA-induced changes in M1 TH-fiber innervation and monoamine tissue concentrations were quantified using immunohistochemistry and high performance liquid chromatography (HPLC). Next, real-time reverse-transcriptase polymerase chain reaction (PCR) was used to examine changes in M1 gene expression after DA lesion and treatment with L-DOPA or the D_1 receptor agonist SKF81297 (SKF). Our hypothesis was that 6-OHDA would reduce DA and NE innervation of M1, while DA replacement would pathologically enhance expression of M1 immediate-early genes and other genes involved in glutamate signaling, coincident with the induction of dyskinetic behavior.

EXPERIMENTAL PROCEDURES

Animals

All experiments used male Sprague–Dawley rats (Taconic Farms, Hudson, NY, USA) that were 9–11 weeks old at the start of the experiment ($N = 86$). Rats were pair-housed in plastic cages and given free access to water and standard laboratory rat food. The colony room was maintained at 22–23 °C on a 12-h light/dark cycle, with experiments taking place during the light cycle. Throughout the study, animals were cared for in full accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the most-current (2011) National Institutes of Health "Guide for the Care and Use of Laboratory Animals".

Drugs

All drugs were delivered at a volume of 1 ml/kg. Buprenorphine hydrochloride (Hospira Inc., Lake Forest, IL, USA) was dissolved in saline. (±)SKF hydrobromide (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in saline with 20% dimethyl sulfoxide. 6-OHDA hydrobromide and L-DOPA methyl ester hydrochloride (Sigma–Aldrich) were dissolved in saline with 0.1% ascorbic acid. Multiple doses of L-DOPA were used, but the peripheral decarboxylase inhibitor benserazide hydrochloride (Sigma–Aldrich) was always co-administered at a constant dose of 15 mg/kg. Sodium pentobarbital (Fort Dodge Animal Health, Fort Dodge, IA, USA) was suspended in an alcohol/dH_2O mixture by the manufacturer. Desipramine hydrochloride and quinpirole hydrochloride (Sigma–Aldrich) were dissolved in dH_2O.

Lesion surgeries

All rats received unilateral lesions to the medial forebrain bundle (MFB). In different experiments, sham or active lesions were created by infusing vehicle (Veh) or 6-OHDA, respectively. For analgesic purposes, rats were given buprenorphine (0.03 mg/kg; i.p.) immediately prior to surgery and 24 h after surgery. Animals were anesthetized with isoflurane (2–3% for 30–45 min; Baxter Healthcare, Deerfield, IL, USA) mixed with oxygen (1 L/min). Since PD patients show significant NE pathology in M1 (Gaspar et al., 1991), NE neurons were not protected from pathology using the NE transport blocker desipramine. The exception to this is experiment 3B, where rats were treated with desipramine (25 mg/kg, ip) 30 min prior to 6-OHDA infusion, since these rats were used as part of a different study (see experiment 1 of Dupre et al., 2013). The following coordinates relative to the bregma were used to target the MFB according to the rat brain atlas of Paxinos and Watson (1998): AP −1.8 mm; ML −2.0 mm; DV −8.6 mm, with the incisor bar 5 mm below the interaural line. A syringe with 26-gauge needle (Hamilton, Reno, NV, USA) was lowered into the target site and 6-OHDA (12 μg) or Veh was injected at a constant flow rate of 2 μl/min for 2 min. The needle was withdrawn 5 min later.

Forepaw adjusting steps (FAS) test

The FAS test is a measure of akinesia, a cardinal symptom of PD (Jankovic, 2008). Rats with >80% unilateral striatal DA depletion take fewer steps with the lesioned side of the body (Chang et al., 1999). To perform the test, an experimenter blinded to treatment condition held the rat's hindlimbs and one forelimb such that the free forelimb was forced to bear the rat's body weight. Rats were moved laterally for 90 cm over 10 s while another experimenter