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Research Paper

Striatal cholinergic interneurons and D2 receptor-expressing GABAergic medium spiny neurons regulate tardive dyskinesia

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ABSTRACT

Tardive dyskinesia (TD) is a drug-induced movement disorder that arises with antipsychotics. These drugs are the mainstay of treatment for schizophrenia and bipolar disorder, and are also prescribed for major depression, autism, attention deficit hyperactivity, obsessive compulsive and post-traumatic stress disorder. There is thus a need for therapies to reduce TD. The present studies and our previous work show that nicotine administration decreases haloperidol-induced vacuous chewing movements (VCMs) in rodent TD models, suggesting a role for the nicotinic cholinergic system. Extensive studies also show that D2 dopamine receptors are critical to TD. However, the precise involvement of striatal cholinergic interneurons and D2 medium spiny neurons (MSNs) in TD is uncertain. To elucidate their role, we used optogenetics with a focus on the striatum because of its close links to TD. Optical stimulation of striatal cholinergic interneurons using cholineacetyltransferase (ChAT)-Cre mice expressing channelrhodopsin2-eYFP decreased haloperidol-induced VCMs (~50%), with no effect in control-eYFP mice. Activation of striatal D2 MSNs using Adora2a-Cre mice expressing channelrhodopsin2-eYFP also diminished antipsychotic-induced VCMs, with no change in control-eYFP mice. In both ChAT-Cre and Adora2a-Cre mice, stimulation or mecamylamine alone similarly decreased VCMs with no further decline with combined treatment, suggesting nAChRs are involved. Striatal D2 MSN activation in haloperidol-treated Adora2a-Cre mice increased c-Fos$^+$ D2 MSNs and decreased c-Fos$^+$ non-D2 MSNs, suggesting a role for c-Fos. These studies provide the first evidence that optogenetic stimulation of striatal cholinergic interneurons and GABAergic MSNs modulates VCMs, and thus possibly TD. Moreover, they suggest nicotinic receptor drugs may reduce antipsychotic-induced TD.

1. Introduction

Antipsychotics are a very important class of drugs approved for the management of schizophrenia and bipolar disorder, and used off-label for depression, autism, attention deficit hyperactivity disorder, obsessive compulsive disorder and post-traumatic stress disorder (Canitano and Scandurra, 2011; Pickar et al., 2008; Seeman, 2010; Tarsy et al., 2011). These dopamine receptor antagonists are thought to exert their therapeutic effect by dampening dopaminergic activity (Seeman, 2010; Tarsy et al., 2011; Turrone et al., 2003). However, their continued use also results in motor side effects including tardive dyskinesia (TD) that afflicts up to 25% of treated patients (Saha et al., 2005).

TD are drug-induced repetitive abnormal involuntary movements primarily of the face and limbs that range from troubling to debilitating (Correll et al., 2004; Tarsy et al., 2011). The more recently developed second-generation antipsychotics produce less TD; however, it still arises (Correll and Schenk, 2008; Peluso et al., 2012; Tarsy et al., 2011; Woods et al., 2010). Little treatment is currently available for TD other than antipsychotic dose modification. There is thus a need for novel approaches to attenuate its occurrence. Our previous work had shown that nicotine treatment reduced haloperidol-induced abnormal movements or vacuous chewing movements (VCMs) in a rat model of TD, with a maximal reduction of 50% (Bordia et al., 2012). These findings suggest that drugs modulating the nicotinic cholinergic system may minimize TD in patients requiring antipsychotics.

The neuronal cell subtypes that contribute to TD are currently uncertain because CNS circuitry is complex and tightly integrated with numerous other neuronal systems. The striatum may be involved since it is a key region in the control of normal movement (Gittis and Kreitzer, 2012; Teo et al., 2012) and aberrant motor behaviors that arise with antipsychotics (Loonen and Ivanova, 2013). Neurotransmitter systems in the striatum that may play a role include the cholinergic one (Deffains and Bergman, 2015; Kelley and Roberts, 2004; Lovinger, 2010). Cholinergic interneurons have extensive, dense axonal arbors that are very widespread throughout the striatum despite the fact that they comprise

Abbreviations: ANOVA, analysis of variance; TD, tardive dyskinesia; nAChR, nicotinic acetylcholine receptor; *, the asterisk indicates the possible presence of other subunits in the nicotinic receptor complex.

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only 2–3% of the total number of striatal neurons. Moreover, striatal cholinergic interneurons are associated with motor control and cholinergic agonists such as nicotine and varenicline decrease haloperidol-induced VCMs (Bordia et al., 2013; Quik et al., 2014). Indirect pathway GABAergic medium spiny neurons (MSNs) are likely also involved as they express D2 receptors, and antipsychotics are thought to act by blocking D2 receptors, possibly via a compensatory receptor up-regulation in response to chronic antipsychotic treatment (Seeman, 2010; Turrone et al., 2003).

The objective of the present studies was to elucidate the role of striatal cholinergic interneurons and D2 MSNs in TD using optogenetics. This approach offers the advantage that it permits in vivo cell type specific targeting of select neuronal populations in real time. To investigate the role of the striatal cholinergic system, we used cholineacetyl transferase (ChAT)-Cre mice that allows for the selective expression and activation of channelrhodopsin2 (ChR2) in striatal cholinergic interneurons. To elucidate the involvement of striatal D2 receptor-expressing GABAergic MSNs (D2 MSNs), we used ChR2-expressing Adora2a-Cre mice in which D2 MSNs can be selectively activated. The data indicate that both cholinergic interneurons and D2 MSNs regulate TD.

2. Materials and methods

2.1. Animals

All procedures conformed to the guidelines mandated by the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the SRI Institutional Animal Care and Use Committee.

2.2. Studies with control mice

Fifty adult (18–20 g) male C57 BL/6 mice (Charles River, Livermore, CA) were housed 3–4 per cage and quarantined for 2 days under a 12/12 h light-dark cycle in a temperature and humidity-controlled room with free access to food and water. Several days later, all mice were acclimated to 2% saccharin in drinking water. They were then randomly assigned to saccharin with (n = 25) or without (n = 25) nicotine (free base; Sigma-Aldrich Co., St. Louis, MO), with saccharin used to mask the bitter taste of nicotine. Nicotine was started at 25 μg/ml and incrementally increased (50, 100, 200 μg/ml) over 10 days to a final concentration of 300 μg/ml, a dose that yields plasma cotinine levels of 200–250 ng/ml (Huang et al., 2011; Quik et al., 2012). The mice were weighed once weekly, with no significant difference between nicotine and saccharin-treated groups.

After 2 weeks. of saccharin/nicotine treatment, one set of mice was injected with vehicle or haloperidol (1 mg/kg intraperitoneally; Sigma-Aldrich Co., St. Louis, MO) twice daily (5 days per week). Another set of mice was surgically implanted with blank pellets or pellets releasing 3 mg/kg/day haloperidol (Innovative Research of America, Sarasota, FL, USA) over 90 days, after which they were replaced if necessary. The pellets were inserted under isoflurane anesthesia into the subcutaneous space on the dorsal surface (Crowley et al., 2012). Mouse weights were similar between treatments.

To rate VCMs, each mouse was placed in a circular Plexiglas chamber devoid of bedding. For the injection group, mice were allowed to acclimate in the cylinder for 2 min, injected with haloperidol and immediately rated for the number of VCMs in a 5 min period by a blinded rater. VCMs consisted of subtle chewing movements, high frequency jaw/mouth tremors and purposeless mouth openings in the vertical plane with or without tongue protrusion (Blanchet et al., 2012; Bordia et al., 2012; Turrone et al., 2002). For the pellet-treated group, mice were allowed to adapt to the cylinder for 2 min and rated. VCMs were assessed from the start of the study but only developed by 7 weeks of haloperidol treatment, with the data provided for 7 weeks and onwards.

2.3. Studies with genetically modified mice

Homozygous male ChAT-internal ribosome entry site-cre knockin mice were purchased from Jackson Laboratory (B6.129S6-Chat$^{tm1(cre)Lowl}$). Heterozygous male Adora2a-Cre indirect pathway (striatopallidal, D2) specific BAC transgenic mice (KG139Gsat/Mmcd, B6.FVB(Cg) background) were obtained from MMRC and bred in-house.

After acclimation, mice were bilaterally injected with cre-inducible recombinant AAV vector expressing ChR2 (AAV5.EF1a.DIO.hChR2(H134R)-eYFP.WPRE.hGH) or control vector (AAV5.EF1a.DIO.eYFP.WPRE.hGH) (Freeze et al., 2013; Threlfell et al., 2012). Virus (1 μl at 1–4 × 10^{12} particles/ml), obtained from the Vector Core, Univ Pennsylvania, was injected bilaterally into the striatum at: AP +0.8 mm, ML ±2.2 mm, DV −2.7 mm. Immediately after injection, the mice were implanted bilaterally with optical fibers (0.2 mm diameter) in the dorsolateral striatum (AP +0.8 mm, ML ±2.2 mm, DV −2.5 mm). Four weeks after virus injection, the mice were implanted with pellets releasing haloperidol (3 mg/kg/day).

Eight weeks after pellet implantation, the effect of optical illumination was determined, as described (Bordia et al., 2016). ChR2 was activated using a 473 nm diode laser adjusted such that the power was 1 mW at each fiber tip. Light intensity at 0.2 mm from the tip was calculated to be 15.9 mW/mm^2 (corresponding to 73.2 mW/mm^2 at the fiber tip) (http://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php). Optical stimulation consisted of a single pulse (2 ms to 1 s in duration) or of various burst paradigms, as described (Cachope et al., 2012; Freeze et al., 2013; Threlfell et al., 2012), with a 0.5 s off stimulation period between pulses or bursts. Stimulation was for 5 min, during which time VCMs were scored. In some studies, the general nicotinic antagonist mecamylamine (1 mg/kg subcutaneously; Sigma-Aldrich Co., St. Louis, MO) was administered. For these experiments, each mouse was first rated without stimulation for 5 min. Mecamylamine was then injected and 30 min later the mouse was again optically stimulated and rated. The effect of mecamylamine was evaluated 30 min after injection consistent with previous work (Bordia et al., 2010; Bordia et al., 2016; Dekundy et al., 2007).

2.4. Immunohistochemistry

Mice were killed by cervical dislocation and immunohistochemistry done as described (Bordia et al., 2016). The brains were quickly removed, fixed in 4% paraformaldehyde for 2–3 days and cryopreserved in 10–30% sucrose for 3 days. They were sectioned (30 μm) using a cryostat (Leica). Every sixth section throughout the striatum (A1.4 to A −0.4) (total ~ 12 sections/mouse) was stained for ChAT, DARPP-32 or c-Fos. Free floating sections were rinsed, blocked and then incubated overnight at 4 °C with goat anti-ChAT (1:200 dilution; Millipore), rabbit anti-DARPP-32 (1:1000, Cell Signaling Technology) or rabbit anti-c-Fos (1:600 dilution; Cell Signaling Technology). They were next incubated for 2 h at room temperature with secondary antibody (donkey anti-goat Alexa-568; dilution 1:200; Invitrogen: or goat anti-rabbit Alexa-555; dilution 1:500; Cell Signaling Technology). Sections were rinsed, mounted onto slides using Vectashield mounting medium (Vector Labs), coverslipped and viewed under a Nikon fluorescence microscope (model Eclipse E400).

To view viral spread in striatal neurons, images were captured at 4× magnification for e-YFP expression. For cell counting analysis, images from the dorsolateral striatum were taken at 40× magnification. Expression of ChR2-eYFP in cholinergic interneurons was evaluated by counting all ChAT/e-YFP double positive cells. To delineate ChR2-eYFP expression in D2-MSNs, DARPP-32 and e-YFP double positive cells were obtained by merging individual signals using Adobe Photoshop software, as described (Bordia et al., 2016). The proportion of ChR2-eYFP positive D2-MSNs was obtained by calculating the ratio of the number of double positive cells to the total number of DARPP-32 expressing cells in a blinded manner. c-Fos$^+$ cell counting was done at