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Adenosine A2a receptor antagonists attenuate striatal adaptations following dopamine depletion

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ABSTRACT

The motor symptoms of Parkinson's disease (PD) are widely thought to arise from an imbalance in the activity of the two major striatal efferent pathways following the loss of dopamine (DA) signaling. In striatopallidal, indirect pathway spiny projection neurons (iSPNs), intrinsic excitability rises following the loss of inhibitory D2 receptor signaling. Because these receptors are normally counterbalanced by adenosine A2a adenosine receptors, antagonists of these receptors are being examined as an adjunct to conventional pharmacological therapies. However, little is known about the effects of sustained A2a receptor antagonism on striatal adaptations in PD models. To address this issue, the A2a receptor antagonist SCH58261 was systemically administered to DA-depleted mice. After 5 days of treatment, the effects of SCH58261 on iSPNs were examined in brain slices using electrophysiological and optical approaches. SCH58261 treatment did not prevent spine loss in iSPNs following depletion, but did significantly attenuate alterations in synaptic currents, spine morphology and dendritic excitability. In part, these effects were attributable to the ability of SCH58261 to blunt the effects of DA depletion on cholinergic interneurons, another striatal cell type that co-expresses A2a and D_2 receptors. Collectively, these results suggest that A2a receptor antagonism improves striatal function in PD models by attenuating iSPN adaptations to DA depletion.

Introduction

The motor symptoms of PD have long been thought to be the product of an imbalance in the so-called direct and indirect striatal output pathways, leading to sustained inhibition of the motor thalamus and difficulty in initiating movement (Albin et al., 1989). The best evidence for this hypothesis comes from studies showing that excitability of indirect pathway, striatopallidal spiny projection neurons (iSPNs), is increased following DA depletion. *In vivo*, the spontaneous activity and synaptic responsiveness of antidromically-identified iSPNs is elevated following depletion (Ballion et al., 2009; Mallet et al., 2006) and reducing their activity ameliorates the motor symptoms associated with DA depletion (Kravitz et al., 2010). In brain slices from depleted mice, dendritic excitability is elevated in iSPNs and plasticity at excitatory glutamatergic synapses becomes biased toward long-term potentiation (LTP) (Day et al., 2006; Kreitzer and Malenka, 2007; Malenka and Bear, 2004; Shen et al., 2008). This elevated excitability triggers a form of homeostatic plasticity (Turrigiano, 2007), leading to a dramatic loss in iSPN excitatory synaptic connections and spines in the days following DA depletion (Day et al., 2006), partially disconnecting the cerebral cortex from the striatum.

Diminishing the change in iSPN excitability following DA depletion should attenuate these adaptations and ameliorate motor impairment. Indirect and direct DA receptor agonists are commonly used to this end, but they often have unwanted side-effects or limited efficacy (Jenner, 2003b). Adenosine A2a receptor (A2aR) antagonists are being examined as potential adjuncts to DA receptor based therapies (Hauser and Schwarzschild, 2005; Jenner, 2003a; Pinna et al., 2010). A2aRs are expressed primarily in the striatum where they are found in iSPNs and cholinergic interneurons, both of which also robustly express D_2 receptors (D2Rs) (Hauser and Schwarzschild, 2005; Preston et al., 2000; Song et al., 2000; Surmeier et al., 2009). These two signaling pathways negatively interact at several levels (Canals et al., 2004; Morelli et al., 2007; Svenningsson et al., 1999), suggesting that following DA depleting lesions, not only does D2R signaling fall, but A2aR signaling also rises. This antagonism also suggests that an elevation in A2aR signaling should reduce the ability of residual DA to effectively modulate iSPNs (Fuxe et al., 2007). In agreement with this view, in human patients with PD, it has been hypothesized that A2aR antagonists will decrease the amount of levodopa required to achieve symptomatic relief and, in so doing, decrease dyskinesias (Hauser and Schwarzschild, 2005; Jenner, 2003a; Morelli et al., 2007). In animal models, A2aR antagonists have similar effects (Hauser and Schwarzschild, 2005; Jenner, 2003a; Morelli et al., 2007).

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Beyond the immediate effects on cellular excitability to be expected by potentiating residual D2R signaling, it is not clear what antagonizing A2aRs will achieve. Considering recent work (Shen et al., 2008), A2aR antagonists should diminish biasing of synaptic plasticity at glutamatergic synapses toward LTP after DA depletion. This should, in principle, decrease the responsiveness of iSPNs to cortical activity, helping to bring iSPN activity back toward normal levels. However, it remains to be determined whether sustained DA depletion *in vivo* leads to the potentiation of corticostriatal glutamatergic synapses on iSPNs. Furthermore, it is far from clear whether all the effects of A2aR antagonism are directly mediated by actions on iSPNs. As mentioned above, A2aRs and D2Rs are also co-expressed by cholinergic interneurons and these interneurons exert a powerful effect on iSPNs and the striatal circuitry (Pisani et al., 2007; Tozzi et al., 2011; Wang et al., 2006).

This study was undertaken to provide answers to some of these questions. Our studies show that although the A2aR antagonist SCH58261 did not prevent the loss of spines and synapses following DA depletion, it did significantly reduce other adaptations in iSPNs. Moreover, the SCH58261 effect on at least one of these adaptations – an increase in dendritic excitability – appeared to be mediated indirectly through cholinergic interneurons.

Materials and methods

Behavior

Catalepsy responses were measured by means of the bar method. The forepaws of the mice were placed over a steel horizontal bar, 0.5 cm in diameter and 10 cm long, fixed at a height of 3 cm above the working surface. The duration of catalepsy was measured as the time from forepaw placement on the bar until the mice removed both forepaws from the bar or climbed over the bar with the hind limbs. Mice were given three trials. Total time was calculated as the sum of the 3 trials with a maximum time of 15 min. The bar test was performed 4 h after drug administration to coincide with the time of electrophysiology and Ca^{2+} imaging experiments that were performed on the fifth day of drug administration 4 h after the last injection.

6-hydroxydopamine (6-OHDA) DA depletion

6-OHDA DA depletion was produced by a unilateral lesion of the nigrostriatal system by 6-OHDA injection into the medial forebrain bundle (MFB). In brief, mice at postnatal days 23–28 were anesthetized with a SurgiVet isoflurane vaporizer (Smiths Medical PM, Inc., Norwell, MA). After immobilization on a stereotaxic frame (Model 940, David Kopf Instruments, Tujunga, CA) with a Cunningham adaptor (Harvard Apparatus, Holliston, MA), a hole was drilled (~1 mm diameter) at 0.7 mm posterior and 1.1 mm lateral to bregma for injection into the MFB (0.7 AP = anterior–posterior, 1.1 ML = medial–lateral, 4.8 DV = dorsal–ventral). 1 μl of 6-OHDA HCl was dissolved at a concentration of 3.5 μg/μl saline with 0.02% ascorbate and injected using a calibrated glass micropipette (2-000-00, Drummond Scientific Company, Broomall, PA), at a depth of 4.8 mm from the surface of the skull, conducting at a rate of 0.02 μl/min. The micropipette was left *in situ* for another 30 min after the injection to maximize tissue retention of 6-OHDA and decrease capillary spread upon pipette withdrawal. Electrophysiological experiments were performed 3–4 weeks later.

Brain slice preparation

Horizontal brain slices (275 μm) were obtained from 27 to 37 day old BAC D2 or D1 transgenic mice following procedures approved by the Northwestern University Animal Care and Use Committee and guidelines of the National Institutes of Health. The mice were anesthetized with isoflurane (Baxter, Deerfield, IL) and decapitated. Brains were rapidly removed and sectioned in oxygenated, ice-cold, artificial cerebral spinal fluid (ACSF) using a Leica VT1000S vibratome (Leica Microsystems, Wetzlar, Germany). The ACSF contained the following (in mM): 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1 NaH_2PO_4, and 16.7 D-(+)-glucose. The slices were transferred to a holding chamber where they were incubated in ACSF at 35 °C for 30 min, after which they were stored at room temperature until whole-cell recording experiments (1–5 h). The external ACSF solutions were bubbled with 95% O_2/5% CO_2 at all times to maintain oxygenation and a pH ≈ 7.4. The solutions were periodically checked and adjusted to maintain physiological osmolality (300 mOsm/l).

Electrophysiology

Whole-cell voltage-clamp recordings were obtained using standard techniques. Slices were transferred to a submersion-style recording chamber mounted on an Olympus BX51-WI upright, fixed-stage microscope (Melville, NY). The slices where continuously perfused with oxygenated 1.5 ml/min ACSF at room temperature. For strontium excitatory postsynaptic current (strEPSC) voltage-clamp experiments, pipettes (3–5 MΩ) were filled with a Cs^+ internal solution containing the following (in mM): 120 $CsMeSO_3$, 15 CsCl, 8 NaCl, 10 TEA-Cl, 10 HEPES, 2–5 QX-314, 0.2 EGTA, 2 Mg-ATP, 0.3 Na-GTP, pH 7.3 adjusted with CsOH, and were performed at room temperature. Ca^{2+} was replaced with Sr^{2+} and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) mediated quantal events (strEPSCs) were collected during a 300-ms period beginning 50 ms following each stimulus (delivered once every 30 s) in an external ACSF solution containing 50 μM D-APV, 10 μM (−) SR95531 (gabazine), 2 mM Sr^{2+} and 0 Ca^{2+}. Miniature events were analyzed using Minianalysis software (Synaptosoft, Decatur, GA) with detection parameters set at greater than 5 pA amplitude and individually verified. For each cell, at least 300 strEPSCs were taken for constructing cumulative probability plots and calculating mean strEPSC amplitudes and frequencies. Stimulation (50–200 μs) was performed using steel concentric electrodes (Frederick Haer & Co, Bowdoin, ME). The corticostriatal afferents were stimulated by placing the stimulation electrode between layer V and VI in the cortex (Fig. 2A).

2-photon laser scanning microscopy (2PLSM), Ca^{2+} imaging, and spine morphology

Slices were transferred to a submersion-style recording chamber mounted on an Olympus BX51-WIF upright, fixed-stage microscope (Melville, NY). The slices where continuously perfused with oxygenated 1.5 ml/min ACSF at room temperature. D2 (BAC D2) receptor expressing iSPNs were identified by somatic eGFP two photon excited fluorescence using an Ultima Laser Scanning Microscope (2PLSM) system (Prairie Technologies, Middleton, WI). A DODT contrast detector system was used to provide a bright-field transmission image in registration with the fluorescent images. The green GFP signals (490–560 nm) were acquired using 810 nm excitation (Verdi/Mira laser: Coherent Laser Group, Santa Clara, CA). iSPNs were patched using video microscopy with a Hitachi CCD camera (model KP-M2RN, Tokyo, Japan) and an Olympus *UIS1* 60×/0.9 NA water-dipping lens. Patch electrodes were made by pulling BF150-86-10 glass on a P-97 Flaming/Brown micropipette puller (Sutter Instrument Co., Novato, CA). The pipette solution contained the following (in mM): 135 $KMeSO_4$, 5 KCl, 5 HEPES, 2 MgATP, 0.5 Na_2GTP, 10 phosphocreatine-Na, pH = 7.25–7.3 with KOH, 270 mOsm/l. Electrophysiological recordings were obtained with a Multiclamp 700B amplifier (Molecular Devices, Inc., Sunnyvale, CA) and then digitized with the scanning computer (PCI MIO-16E-4, National Instruments, Austin, TX). The stimulation, display and analysis software was a custom-written shareware package, WinFluor (John Dempster, Strathclyde University, Glasgow, Scotland; UK). WinFluor automated and synchronized the two-photon excited fluorescence with the