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Alternative splicing of AMPA receptor subunits in the 6-OHDA-lesioned rat model of Parkinson's disease and L-DOPA-induced dyskinesia

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ABSTRACT

Abnormal corticostriatal plasticity is a key mechanism of L-DOPA-induced dyskinesia (LID) in Parkinson's disease (PD). Antagonists at glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, such as IEM 1460, reduce induction and expression of dyskinesia in rat and non-human primate models of PD. AMPA receptor function is regulated by post-transcriptional splicing of subunit mRNA to produce *flip* and *flop* isoforms, which may therefore influence corticostriatal plasticity. The aim of this work was to evaluate alterations in alternative splicing of striatal AMPA receptor subunits in the unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model of LID and PD. Male Sprague–Dawley rats received 12.5 μg 6-OHDA injections into the right medial forebrain bundle. In experiment 1, to assess acute dyskinesia, rats received L-DOPA/benserazide (6/15 mg/kg, i.p.) or vehicle for 21 days. In experiment 2, to assess dyskinesia priming, rats received vehicle, L-DOPA + vehicle or L-DOPA + IEM 1460 (3 mg/kg, i.p.) for 21 days. Animals were humanely killed 1 h following final treatment in experiment 1, and 48 h following final treatment in experiment 2. Coronal sections of rostral striatum were processed for *in situ* hybridisation histochemistry, using oligonucleotide probes specific for the GluR1 and GluR2 subunits and their flip and flop isoforms. L-DOPA treatment increased GluR2-flip mRNA expression in the lesioned striatum of both groups; this was blocked by the Ca^{2+}-permeable AMPA receptor antagonist IEM 1460. GluR1-flip expression was increased after 48 h drug washout but not in acute LID. There were no changes in expression of flop isoforms. Alternative splicing of AMPAR subunits contributes to abnormal striatal plasticity in the induction and expression of LID. Increases in GluR2-flip expression depend on activation of Ca^{2+}-permeable AMPA receptors, which are a potential target of anti-dyskinetic therapies.

Introduction

L-DOPA-induced dyskinesia (LID) is a common complication of chronic dopaminergic treatments for Parkinson's disease (PD) (Ahlskog and Muenter, 2001). Pathologically-enhanced corticostriatal plasticity appears to be key to the development and subsequent expression of LID, and glutamate receptors are implicated in this process (Chase et al., 2003; Picconi et al., 2003). Antagonists at ionotropic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors, as well as metabotropic glutamate receptors, have been shown to reduce established LID in animal models of PD (Blanchet et al., 1998; Dekundy et al., 2006; Konitsiotis et al., 2000).

AMPA receptors are composed of combinations of GluR1-4 subunits in a tetrameric configuration (Keinanen et al., 1990). The subunit structure is common to other members of the ionotropic glutamate receptor family, with an extracellular N-terminal domain, re-entrant transmembrane domain and a large extracellular portion between the second and third transmembrane domains (Mayer and Armstrong, 2004). Within the striatum, GluR1 and GluR2 subunits are the most abundant (Deng et al., 2007; Stefani et al., 1998).

Post-transcriptional modification of AMPA receptor subunits contributes significantly to regulation of receptor function. RNA editing of the GluR2 subunit at codon 607 renders GluR2-containing AMPA receptors impermeable to calcium (Burnashev et al., 1996). Post-transcriptional alternative splicing of AMPA receptor subunits at the second extracellular domain gives rise to *flip* and *flop* splice variants (Sommer et al., 1990). This process modifies the pharmacokinetic properties of the ion channel, such that *flip* splice variants desensitise more slowly than *flop* isoforms, and produce greater synaptic currents (Koike et al., 2000; Mosbacher et al., 1994). Alternative splicing of central AMPA receptor subunits occurs in a region-dependent manner (Sommer et al., 1990), and altered expression of splice variants has

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been reported in neurological conditions including epilepsy and motor neuron disease (Seifert et al., 2002; Tomiyama et al., 2002).

As well as reducing established dyskinesia, AMPA receptor antagonists have been shown to attenuate the process of priming for LID in animal models of PD (Kobylecki et al., 2010; Konitsiotis et al., 2000). In particular, recent work implicates GluR2-lacking, Ca^{2+}-permeable AMPA receptors in both of these processes (Kobylecki et al., 2010). Studies in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of PD demonstrated that the anti-dyskinetic action of AMPA receptor antagonists is mediated at the striatum (Maranis et al., 2012). However, the molecular mechanisms of AMPA receptor overactivity in LID are not fully understood. Increased AMPA receptor binding has been identified in the striatum of the dyskinetic MPTP-lesioned nonhuman primate (NHP), and in dyskinetic patients with PD (Calon et al., 2002, 2003). Alterations in subcellular trafficking and phosphorylation of AMPA receptor subunits have also been described in animal models of LID (Santini et al., 2007; Silverdale et al., 2010). The role of alternative splicing has not been explored in these models, although an increase in GluR2-*flip* mRNA expression in the lateral striatum following chronic haloperidol treatment suggests its importance in control of glutamate-dopamine interactions in the basal ganglia (Brene et al., 1998). Given the effects of alternative splicing on the pharmacological properties of AMPA receptors, we hypothesised that alterations in striatal expression of *flip* and *flop* isoforms could contribute to abnormal corticostriatal plasticity in LID. If alterations in AMPA receptor splicing contribute to dyskinesia priming, they should be affected by modulation of neurochemical pathways involved in priming. Given the contribution of Ca^{2+}-permeable AMPA receptors to early phases of synaptic plasticity, we hypothesised that blockade of this pathway would prevent the later upregulation of AMPA receptor splice variants in the primed state.

We report a study of alterations in splicing of the GluR1 and GluR2 subunits in the striatum of L-DOPA-treated unilateral 6-hydroxydopamine (6-OHDA)-lesioned rat model of PD. Increased expression of GluR2-*flip* mRNA was present in the lateral striatum of animals expressing abnormal involuntary movements (AIMs), the rat homolog of LID. These results led us to investigate the role of AMPA receptor splicing in dyskinesia priming. These experiments show that increases in *flip* isoforms of GluR1 and GluR2 are also involved in the process of priming for LID, and are prevented by blockade of Ca^{2+}-permeable AMPA receptors.

Methods

All animal studies were conducted in accordance with the U.K. Animals (Scientific Procedures) Act 1986.

Unilateral 6-OHDA lesion surgery

Male Sprague–Dawley rats (280–310 g; Charles River, U.K.) were maintained at constant temperature and humidity on a 12 hour light/dark cycle, and allowed free access to food and water. Thirty minutes before surgery, rats received pargyline (5 mg/kg, i.p.; Sigma-Aldrich, U.K.) and despiramine (25 mg/kg, i.p.; Sigma-Aldrich) dissolved in 1 ml/kg sterile saline. 6-OHDA hydrobromide (Sigma-Aldrich) was dissolved in ice-cold sterile water (5 mg/ml); 2.5 μl was injected over 5 min using a Hamilton syringe into the right medial forebrain bundle under isoflurane anaesthesia. The needle was left in place for a further 5 min and then withdrawn. Stereotaxic coordinates, in mm relative to bregma, were: A = −2.8, L = −2.0, and V = −8.6 (Paxinos and Watson, 1998).

Behavioural analysis

Animals were screened for inclusion into the study at three weeks post-lesion, using the cylinder test, as previously described (Kirik et al., 2000). Animals with a cylinder test performance of <30%, corresponding to >90% dopamine depletion (Lundblad et al., 2004), were included in the study.

Abnormal involuntary movements (AIMs) were assessed as previously described, with modifications (Kobylecki et al., 2010; Lundblad et al., 2002). Animals were placed in clear Perspex boxes and allowed to acclimatise for 30 min prior to the first behavioural assessment. AIMs were assessed in one-minute sessions for each animal at 30-minute intervals, for a total of 180 min following drug administration. The severity of axial, limb and orolingual (ALO) AIMs was scored from 0 to 4, giving a maximal ALO AIM score per rating session of 72. Locomotor AIMs were assessed separately, as previously reported.

Drug treatment

L-DOPA methyl ester hydrochloride and benserazide (both Sigma-Aldrich) were dissolved in 1 ml/kg sterile saline. IEM 1460 (Tocris Bioscience, U.K.) was dissolved in 1 ml/kg sterile water and administered 15 min before L-DOPA.

Experimental design

Experiment 1: Alternative splicing of AMPA receptor subunits in acute dyskinesia. 6-OHDA-lesioned rats were treated for 21 days with daily L-DOPA/benserazide (6/15 mg/kg, i.p.; n = 7) or vehicle (n = 5), commencing five weeks post-lesion. AIMs were assessed on day 21. On day 22, animals received one further treatment with L-DOPA/benserazide or vehicle, before being humanely killed 1 h post-dose by exposure to rising concentrations of CO_2 followed by cervical dislocation. The extent of 6-OHDA lesion in this group was determined by binding of the selective dopamine transporter ligand [^{125}I]-RTI-121, as previously described (Staley et al., 1995). Briefly, air-dried sections were pre-incubated for 30 min with phosphate-buffered saline (PBS; Sigma-Aldrich, U.K.) at room temperature before being incubated with 60 pM [^{125}I]-RTI-121 (Perkin-Elmer, U.K.) at room temperature. Slides were then washed in ice-cold PBS before being dried and apposed to Kodak Biomax MR-1 autoradiographic film (Sigma-Aldrich, U.K.) in the presence of [^{125}I] autoradiographic standards (GE Healthcare Life Sciences, U.K.).

Experiment 2: Alternative splicing of AMPA receptor subunits in dyskinesia priming. 6-OHDA-lesioned rats were treated for 21 days with daily L-DOPA/benserazide (6/15 mg/kg, i.p.; n = 6), L-DOPA/benserazide + IEM 1460 (3 mg/kg, i.p.; n = 7) or vehicle (n = 7), commencing five weeks post-lesion. AIMs were assessed on day 21, and following a final dose of L-DOPA/benserazide in all animals on day 22. On day 24, 48 h following last treatment, animals were humanely killed by exposure to rising concentrations of CO_2 followed by cervical dislocation. The extent of 6-OHDA lesion was determined by high-performance liquid chromatography (HPLC) for dopamine and its metabolites, as previously described (Nash and Brotchie, 2000). Briefly, Monoamines were quantified electrochemically by a dual-carbon electrode high-sensitivity analytical cell (Model 5014B, ESA) coupled to a CoulArray detector (ESA). Chromatograms were recorded and analysed with a chromatographic data system (CoulArray for Windows, Version 3.10, ESA) and quantified by determination of peak areas in relation to standards.

Levels of dopamine, DOPAC and HVA were quantified from concurrently run standard curves and expressed as μg/mg of tissue. Protein content was determined from the pellet remaining after removal of the supernatant for HPLC. Protein standards were run concurrently, and protein content was determined using Bradford reagent (Sigma-Aldrich, UK) on a Lambda-Bio UV/Vis spectrometer (Perkin-Elmer). Behavioural and molecular data from these animals have been previously reported (Kobylecki et al., 2010).