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Pharmacological stimulation of metabotropic glutamate receptor type 4 in a rat model of Parkinson's disease and L-DOPA-induced dyskinesia: Comparison between a positive allosteric modulator and an orthosteric agonist

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ABSTRACT

Metabotropic glutamate receptor 4 ($mGlu_4$) negatively modulates GABA and glutamate release in the 'indirect pathway' of the basal ganglia, and has thus been proposed as a potential target to treat motor symptoms in Parkinson's disease. Here, we present an extensive comparison of the behavioural effects produced by the $mGlu_4$ positive allosteric modulator (PAM), VU0364770, and the $mGlu_4$ orthosteric agonist, LSP1-2111, in rats with unilateral 6-OHDA lesions. The compounds' activity was initially assessed in a test of haloperidol-induced catalepsy in intact rats, and effective doses were then evaluated in the hemiparkinsonian animal model. Neither of the two compounds modified the development of dyskinetic behaviours elicited by chronic treatment with full doses of L-DOPA. When given together with L-DOPA to rats with already established dyskinesias, neither VU0364770 nor LSP1-2111 modified the abnormal involuntary movement scores. VU0364770 potentiated, however, the motor stimulant effect of a sub-threshold L-DOPA dose in certain behavioural tests, whereas LSP1-2111 lacked this ability. Taken together, these results indicate that a pharmacological stimulation of $mGlu_4$ lacks intrinsic antidyskinetic activity, but may have DOPA-sparing activity in Parkinson's disease. For the latter indication, $mGlu_4$ PAMs appear to provide a better option than orthosteric agonists.

1. Introduction

Current pharmacotherapies for Parkinson's disease (PD) aim at ameliorating deficits in central dopaminergic tone. Among these therapies, L-DOPA remains the most effective option (Salat and Tolosa, 2013) but its therapeutic window narrows during the progression of PD, leading to motor fluctuations and abnormal involuntary movements (dyskinesias) (recently reviewed in Cenci et al., 2011; Schneider and Obeso, 2014). These motor complications are perceived as debilitating by the affected patients (Palfreman, 2014) and continue to be a major problem to the clinical management of PD. For this reason, there is a great interest in developing non-dopaminergic treatments that can be added to L-DOPA to reduce these untoward effects (Brotchie, 1998; Cenci et al., 2011; Finlay and Duty, 2014). Moreover, new approaches are being explored to prevent the maladaptive neuroplasticity underlying the long-term development of dyskinesias (Cenci, 2014; Cerasa et al., 2014).

The progressive nigrostriatal degeneration typical of PD leads to an overactivity of the "indirect pathway", which originates from

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dopamine D2 receptor-positive striatal neurons projecting to the external globus pallidus (GPe). The ensuing overactivity of the subthalamic nucleus (STN) plays a critical role in generating parkinsonian motor features (Albin et al., 1989). Accordingly, surgical lesions or functional inactivation of the STN produce marked antiparkinsonian effects (reviewed in Sgambato-Faure and Cenci, 2012).

There is great interest in the possibility of reducing the overactivity of the indirect pathway by targeting specific receptors, such as adenosine A2a receptors (Morelli et al., 2009) or group III metabotropic glutamate receptors (mGlu receptors) (Johnson et al., 2009). Group III mGlus ($mGlu_4$, -7, and -8) can presynaptically inhibit GABA and glutamate release at several nodes of the indirect pathway (reviewed in Conn et al., 2005). Studies based on intracerebroventricular administration of a group III mGlu agonist reported motor stimulant effects in both acute and chronic animal models of PD, and these effects were linked to an inhibitory action on GABAergic transmission at striatopallidal synapses (reviewed in Conn et al., 2005; Finlay and Duty, 2014). A seminal study identified $mGlu_4$ as the crucial mediator of these effects (Valenti et al., 2003). Accordingly, subsequent studies reported that selective agonists of $mGlu_4$ can improve motor deficits in rodent models of PD (Lopez et al., 2007; Niswender et al., 2008; Beurrier et al., 2009).

Until recently, it has been difficult to develop selective agonists for $mGlu_4$, as the orthosteric ligand-binding site of group III mGluR subtypes is highly conserved. On the other hand, mGlus have modulatory sites with less homology between subtypes, the so-called allosteric sites. Highly selective $mGlu_4$ ligands that bind an allosteric modulatory site (positive allosteric modulators, PAMs) have recently been explored as alternative therapeutic options. The earliest $mGlu_4$ PAMs had limited potential for *in vivo* use due to unsuitable chemical properties (Marino et al., 2003). In 2012, the compound, *N*-(3-chlorophenyl)picolinamide (VU0364770) was reported as a systemically active $mGlu_4$ PAM. VU0364770 was found to reverse haloperidol-induced catalepsy when given alone, and exhibited anti-akinetic efficacy when given to unilaterally 6-OHDA-lesioned rats either alone or in combination with subthreshold doses of either the A2a antagonist, preladenant or L-DOPA (Jones et al., 2012). Soon thereafter, additional $mGlu_4$ PAMs, including LuAF21934 and ADX88178 (developed at Lundbeck Pharmaceuticals and Addex Pharmaceuticals, respectively) were reported to alleviate haloperidol-induced catalepsy and potentiate the anti-akinetic effect of L-DOPA (Le Poul et al., 2012; Bennouar et al., 2013).

In parallel with these efforts, Acher and colleagues developed a group III-mGlu orthosteric agonist with preferential activity at $mGlu_4$ (Acher et al., 2007). This compound, termed LSP1-2111, was found to reverse haloperidol-induced catalepsy after intrapallidal administration and to ameliorate deficits in a reaction-time task in rats with partial, bilateral nigrostriatal dopamine lesions (Beurrier et al., 2009). LSP1-2111 was recently evaluated in mice with unilateral 6-OHDA lesions treated with L-DOPA, where it was reported to attenuate the development of dyskinesias upon chronic de novo treatment (Lopez et al., 2011).

The purpose of this study was to determine whether a PAM and an orthosteric agonist at $mGlu_4$ have comparable properties in either potentiating the anti-akinetic action of L-DOPA or attenuating its dyskinesiogenic potential. To this end, we selected VU0364770 and LSP1-2111, well-characterized compounds that are centrally active upon systemic administration (Beurrier et al., 2009; Jones et al., 2012). The compounds were administered to rats with unilateral 6-OHDA lesions, either alone or in combination with L-DOPA, according to different treatment schedules. Our findings suggest that selective potentiation of $mGlu_4$ may provide some mild L-DOPA-sparing activity without inducing dyskinesia.

2. Materials and methods

2.1. Animals

Studies of haloperidol-induced catalepsy were performed at Vanderbilt University using male Sprague–Dawley rats (body weight, 275–300 g) (Harlan Laboratories, Inc Indianapolis, IN). The experimental protocols were approved by the Institutional Animal Care and Use Committee of Vanderbilt University and conformed to the guidelines established by the National Research Council *Guide for the Care and Use of Laboratory*. Studies on hemiparkinsonian animals were performed at Lund University using adult female Sprague–Dawley rats (body weight 225–250 g) (Charles River, Germany), according to our standard procedures (Lindgren et al., 2010; Rylander et al., 2010). The experimental protocols were approved by the Malmö/Lund Ethical Committee on Animal Research. In all studies, rats were housed under 12 h light/dark cycle with free access to food and water. Behavioural tests were performed during the light hours. A total of 240 rats were used in this study (including animals that had to be excluded because of technical failure, and those used in preliminary pharmacokinetic experiments).

2.2. Dopamine denervating lesions

A synopsis of studies using dopamine-denervated rats is provided in Fig. 1. All rats included in these experiments were subjected to a lesion of the right medial forebrain bundle with the neurotoxin 6-hydroxydopamine (6-OHDA-HCl, Sigma Aldrich, Sweden) according to well-standardized procedures (Lindgren et al., 2010; Rylander et al., 2010). Briefly, the toxin was dissolved in 0.02% ascorbate/saline to a concentration of 3.5 μg/μl (free base) and injected at two sites (coordinates given in mm relative to bregma and the dural surface): (1) AP = −4.4, L = −1.2, DV-7.8, with tooth bar at −2.4 (2.5 μl); (2) AP = −4.0, L = −0.8, DV-8.0, tooth bar at +3.4 (2.0 μl). After two weeks, rats were evaluated in the amphetamine-induced rotation test, where the number of rotations was automatically calculated during 90 min after an i.p. injection of dexamphetamine (dexamphetamine-HCl, 2.5 mg/kg, Sigma Aldrich, Sweden, dosed as salt). Rats making more than five full turns/minute in the direction ipsilateral to the lesion were kept for further experiments (Winkler et al., 2002). The amphetamine-induced rotation test resulted in the exclusion of approximately 25% of the 6-OHDA-lesioned animals.

2.3. Drug treatments

All drugs used in these experiments and their administration modalities are reported in Table 1. Drugs were dissolved in the appropriate vehicle (cf. Table 1) immediately prior to use, and administered in a volume of 1 mL/kg; with the exception of VU0364770 which was administered in a volume of 2 mL/kg. L-DOPA was always coadministered with a fixed dose of the peripheral DOPA-decarboxylase inhibitor, benserazide (12 mg/kg, s.c.). VU0364770 and LSP1-2111 were custom synthetized for this study at Vanderbilt Center for Neuroscience Drug Discovery. LSP1-2111 was dissolved in sterile water and administered i.p. according to the procedure published in Beurrier et al. (2009). For VU0364770, several vehicles and routes were tested in preliminary experiments, and peroral drug administration in a DMSO/PEG vehicle was chosen for the dyskinesia studies based both on the pilot results and on our previous experience (Rylander et al., 2010). For the last DOPA-sparing study however, the route was changed to s.c. (with the compound dissolved in 10% Tween vehicle), as in Jones et al. (2012). This change of administration route was prompted by the concern that p.o. dosing may provide slightly lower brain exposure.

2.4. Experimental design

2.4.1. Experiment 1: effect of VU0364770 and LSP1-2111 on haloperidol-induced catalepsy

Rats were administered with haloperidol (1.5 mg/kg, i.p.) 60 min prior to vehicle (10% Tween 80), VU0364770 (1–100 mg/kg, s.c.) or LSP1-2111 (0.3–30 mg/kg i.p.). After an additional 30 min, all rats were assessed for catalepsy. The doses of 100 mg/kg VU0364770 and 15 mg/kg LSP1-2111 produced the largest anti-cataleptic effects, and were thus selected for experiments using 6-OHDA-lesioned rats.

2.4.2. Experiment 2: effect of VU0364770 and LSP1-2111 on already established dyskinesia

The experimental design for experiments 2–4 is outlined in Fig. 1. Two sets of 6-OHDA-lesioned animals received chronic daily treatment with L-DOPA for 3 weeks in order to induce abnormal involuntary movements (AIMs). Dyskinesia rating sessions were carried out 2–3 times per week. Rats that developed moderate/severe and reproducible AIMs were kept for further investigations (11 rats in the study with VU0364770, and 15 rats in the study with LSP1-2111). After the dyskinesia induction phase, rats were kept on a maintenance dosing regimen consisting of 2–4 injections of L-DOPA per week for the rest of the study (Fig. 1A). During this time the rats were acutely challenged with either VU0364770, 100 mg/kg, or LSP1-2111, 15 mg/kg, or their corresponding vehicles, and the L-DOPA-induced AIM scores were rated after each drug challenge. To assess whether the treatments interfered with the therapeutic benefit of L-DOPA (anti-akinetic effects), rats were evaluated also on other motor tests (cylinder test, in the study with VU0364770, and rotarod test in the