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Distinct effects of mGlu4 receptor positive allosteric modulators at corticostriatal *vs.* striatopallidal synapses may differentially contribute to their antiparkinsonian action

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ABSTRACT

Metabotropic glutamate 4 (mGlu4) receptor is a promising target for the treatment of motor deficits in Parkinson's disease (PD). This is due in part to its localization at key basal ganglia (BG) synapses that become hyperactive in this pathology, particularly striatopallidal synapses. In this context, mGlu4 receptor activation using either orthosteric agonists or positive allosteric modulators (PAMs) improves motor symptoms in rodent PD models in certain conditions. However, literature data show that mGlu4 receptor PAMs have no effect at striatopallidal GABAergic synapses (unless combined with an orthosteric agonist) and on the firing activity of pallidal neurons, and fail to provide significant motor improvement in relevant PD models. This questions the mechanistic hypothesis that mGlu4 receptor PAMs should act at striatopallidal synapses to alleviate PD motor symptoms. To shed light on this issue, we performed brain slice electrophysiology experiments. We show that Lu AF21934, an mGlu4 PAM small-molecule probe-compound, was ineffective at striatopallidal synapses at all concentrations tested, while it significantly inhibited corticostriatal synaptic transmission. Similarly, Lu AF21934 did not affect electrophysiology readouts at striatopallidal synapses in the presence of haloperidol or in 6-hydroxydopamine-lesioned rats. Interestingly, co-application of Lu AF21934 with a glutamate transporter inhibitor revealed a significant inhibitory action at striatopallidal synapses. Possibly, this effect could rely on increased level/permanence of glutamate in the synaptic cleft. Such differential efficacy of mGlu4 receptor PAMs at corticostriatal *vs.* striatopallidal synapses raises several issues regarding the synaptic target(s) of these drugs in the BG, and challenges the mechanisms by which they alleviate motor deficits in experimental PD models.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder primarily due to the progressive degeneration of dopaminergic neurons of the *substantia nigra pars compacta* (SNc) projecting to the basal ganglia (BG) (Jankovic, 2008; Olanow, 2007; Schapira and Jenner, 2011). BG consist of a highly organized network of brain nuclei implicated in movement control and motor learning, as well as limbic and cognitive functions, and receive massive projections from several cortical areas. The BG nuclei are constituted of the striatum, which is the main input station, the external *globus pallidus* (GPe or GP in non-primates), the so-called output structures, i.e. the *substantia nigra pars reticulata* (SNr) and the internal *globus pallidus* (GPi or entopeduncular nucleus, EP, in non-primates) that project to the thalamus, and the subthalamic nucleus (STN), which is the second input station and the only intrinsic glutamatergic structure of the BG, the others being mainly GABAergic. Two distinct efferent pathways originate from the striatum and converge to the SNr/GPi: the "direct" pathway, consisting in striatal GABAergic projection neurons expressing D1-like receptors, and the "indirect" pathway, whose neurons express D2-like receptors and project to the SNr/GPi via the GPe and the STN (DeLong and Wichmann, 2007; Levy et al., 1997). In PD, the loss of striatal dopamine leads to two opposite effects: the hypoactivity of the

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direct pathway neurons and, conversely, the hyperactivity of the indirect pathway leading to increased inhibition of the GPe, which in turn disinhibits the STN (Blandini et al., 2000). The overall effect is an increased glutamatergic and a decreased GABAergic tone at the level of the SNr/GPi, which in turn becomes hyperactive and leads to the motor symptoms characterizing PD (DeLong and Wichmann, 2007; Obeso et al., 2000; Wichmann and Dostrovsky, 2011). Moreover, an increased corticostriatal glutamatergic input is thought to contribute to the imbalance of the cortico-BG circuit in PD (Bamford et al., 2004; Chase et al., 2003; Chase and Oh, 2000; Obeso et al., 2008; Yin and Lovinger, 2006).

The gold standard pharmacological treatment for PD motor symptoms is the administration of the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA). However, the relief provided by this therapy is impaired in the long-term by the appearance of dose-dependent side-effects, notably L-DOPA-induced dyskinesia (LID) and motor fluctuations. These have been linked to the pulsatile, non-physiological stimulation of dopamine receptors by the treatment (Ahlskog and Muenter, 2001; Chase, 1998; Olanow et al., 2009). For this reason, novel strategies are now proposed or under investigation and, among them, pharmacotherapy with alternative non-dopaminergic drugs that can relieve PD symptoms by restoring the excitation/inhibition balance in the BG and, at the same time, avoid the development of LID (Brichta et al., 2013; Smith et al., 2012). In this context, the metabotropic glutamate 4 (mGlu4) receptor constitutes a novel and interesting target. This transmembrane G protein-coupled receptor of glutamate (**1**, Fig. 1) is negatively coupled to adenylyl cyclase, thus its activation inhibits voltage-dependent Ca^{2+} channels, resulting in decreased excitability and, particularly, reduced neurotransmitter release (Pin and Acher, 2002). Interestingly, mGlu4 receptors are localized presynaptically on key BG pathways which become hyperactive in PD, in particular at striatopallidal (GABAergic) and corticostriatal (glutamatergic) synapses (Bradley et al., 1999; Calabresi et al., 1993; Conn et al., 2005; Duty, 2010; Galarraga et al., 1987; Gubellini et al., 2002). Accordingly, stimulation of mGlu4 receptors by selective orthosteric agonists provides inhibition of corticostriatal and striatopallidal synaptic transmission *in vitro*, and alleviates motor deficits in rodent PD models (Beurrier et al., 2009b; Cuomo et al., 2009; Valenti et al., 2003). In general, the highly hydrophilic chemical nature of orthosteric agonists or antagonists [amino acid derivatives such as LSP1-2111 (**2**, Fig. 1) or (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG; **3**, Fig. 1)] has been considered a challenge with respect to mGlu receptor sub-type selectivity and blood-brain barrier (BBB) penetration. However, recent efforts within this chemical space yielded compounds with high selectivity (*ca.* 100-fold for mGlu4 receptor against other group III mGluRs)(Flor and Acher, 2012) and unbound compound exposure at the biorelevant phase commensurate with *in vitro* potency, supporting target engagement (Cajina et al., 2013). A complementary strategy to activate these receptors is by using positive allosteric modulators (PAMs). These lipophilic compounds belong in a chemical space better-understood by medicinal chemists, and therefore the perception is that fewer hurdles exist to develop drug candidates with high selectivity and BBB penetration (Conn et al., 2009; Flor and Acher, 2012).

Yet, concerns remain regarding the efficacy of mGlu4 receptor PAMs in alleviating motor deficits in PD. In fact, the two probe compounds reportedly tested using electrophysiology in the GP, namely *N*-phenyl-7-(hydroxylimino)cyclopropa[b]-chromen-1a-carboxamide (PHCCC) (**4**, Fig. 1) (Marino et al., 2003) and VU0155041 (**5**, Fig. 1) (Bogenpohl et al., 2013), failed to produce any significant effect when used alone. These results challenge the hypothesis suggesting the use of such drugs in PD due to their inhibitory action at the striatopallidal synapse (Duty, 2010). Here we attempted to address this issue by testing the mGlu4 receptor PAM Lu AF21934 (**6**, Fig. 1) on corticostriatal *vs*. striatopallidal synapses in rat brain slices by patch-clamp electrophysiology in different conditions.

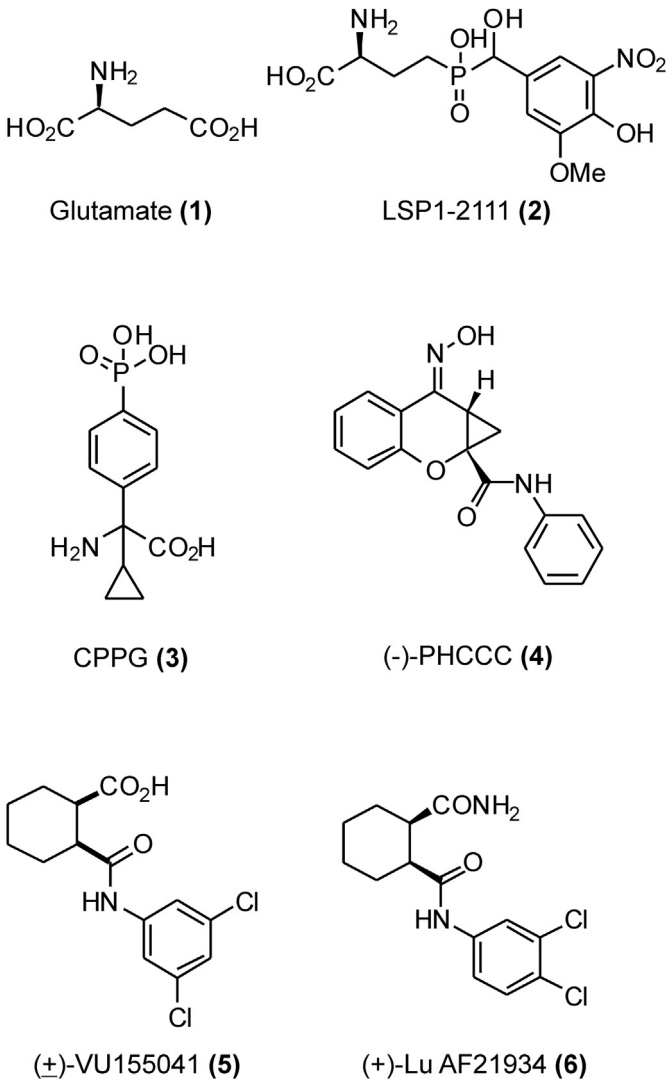

Fig. 1. Chemical structures of glutamate and selected mGlu4 receptor tool compounds.

2. Materials and methods

2.1. Animal care, 6-hydroxydopamine lesion and slice preparation

All animal experiments have been carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and conformed to the ethical guidelines of the French Ministry of Agriculture and Forests (Animal Health and Protection Veterinary Service). All efforts were made to minimize their number and suffering. Male Wistar rats (Charles River, France) were utilized, housed 2 per cage at 20 ± 2 °C in a light controlled environment (12 h light/dark cycle) with access to food and water *ad libitum*.

Surgery for 6-hydroxydopamine (6-OHDA) lesion was performed under Equitesin anesthesia (4 ml/kg) at 6 weeks age. Briefly, rats received a unilateral injection of 12 μg of 6-OHDA (Sigma–Aldrich, France) dissolved in 6 μl of 0.9% sterile NaCl containing 0.1% ascorbic acid, at the rate of 1 μl/min, in the left SNc. The stereotaxic coordinates of the injection site were (from the interaural): anteroposterior 2.2 mm, lateral 2.0 mm, and dorsoventral 3.3 mm, with the incisor bar at +5.0 mm above the interaural plane (De Groot, 1959). To assess the degree of motor impairment produced by dopamine depletion, forelimb-use asymmetry was evaluated using the cylinder test 2 weeks after 6-OHDA lesion (Bennouar et al., 2013; Schallert et al., 2000). Briefly, each rat was placed individually in a Plexiglas® cylinder (30 cm height, 20 cm diameter) and allowed to explore it freely for 20 min, during which time they were filmed in order to be examined later by two independent observers.