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The metabotropic glutamate receptor 8 agonist (*S*)-3,4-DCPG reverses motor deficits in prolonged but not acute models of Parkinson's disease

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ABSTRACT

Metabotropic glutamate receptors (mGlus) are 7 Transmembrane Spanning Receptors (7TMs) that are differentially expressed throughout the brain and modulate synaptic transmission at both excitatory and inhibitory synapses. Recently, mGlus have been implicated as therapeutic targets for many disorders of the central nervous system, including Parkinson's disease (PD). Previous studies have shown that nonselective agonists of group III mGlus have antiparkinsonian effects in several animal models of PD, suggesting that these receptors represent promising targets for treating the motor symptoms of PD. However, the relative contributions of different group III mGlu subtypes to these effects have not been fully elucidated. Here we report that intracerebroventricular (icv) administration of the $mGlu_8$-selective agonist (*S*)-3,4-dicarboxyphenylglycine (DCPG [2.5, 10, or 30 nmol]) does not alleviate motor deficits caused by acute (2 h) treatment with haloperidol or reserpine. However, following prolonged pretreatment with haloperidol (three doses evenly spaced over 18–20 h) or reserpine (18–20 h), DCPG robustly reverses haloperidol-induced catalepsy and reserpine-induced akinesia. Furthermore, DCPG (10 nmol, icv) reverses the long-lasting catalepsy induced by 20 h pretreatment with the decanoate salt of haloperidol. Finally, icv administration of DCPG ameliorates forelimb use asymmetry caused by unilateral 6-hydroxydopamine lesion of substantia nigra dopamine neurons. These findings suggest that $mGlu_8$ may partially mediate the antiparkinsonian effects of group III mGlu agonists in animal models of PD in which dopamine depletion or blockade of D_2-like dopamine receptors is prolonged and indicate that selective activation of $mGlu_8$ may represent a novel therapeutic strategy for alleviating the motor symptoms of PD.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by primary motor symptoms such as resting tremor, rigidity, bradykinesia and postural instability. The major pathophysiological feature of PD is the progressive degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc). This loss of dopaminergic modulation of the striatum alters the control of motor activity by the basal ganglia-thalamo-cortical loop, resulting in the parkinsonian motor symptoms (DeLong and Wichmann, 2007). Current therapeutic strategies to manage and control motor symptoms in PD involve increasing dopaminergic neurotransmission, either by administration of the dopamine precursor L-DOPA or dopamine receptor agonists. Unfortunately, the efficacy of these therapeutic strategies decreases over time in many patients. In addition, long-term L-DOPA therapy is associated with a high incidence of disabling side effects such as dyskinesias and motor fluctuations (Prashanth et al., 2011). As a result, much attention has been focused on identifying novel therapeutic targets

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for PD and metabotropic glutamate receptors (mGlus) have now been implicated as promising new drug targets for alleviating motor symptoms (Conn et al., 2005).

mGlus are 7 Transmembrane Spanning Receptors (7TMRs) that are activated by glutamate, the major excitatory neurotransmitter of the central nervous system (CNS). Eight subtypes of mGlus have been identified to date, and are divided into three groups according to their sequence homology, ligand pharmacology, and downstream signaling pathways (Conn and Pin, 1997). Group I mGlus ($mGlu_1$ and $_{-5}$) signal through $G_{q/11}$ to increase phosphoinositide hydrolysis and mobilize intracellular calcium stores. Group II ($mGlu_2$ and $_{-3}$) and group III ($mGlu_4$, $_{-6}$, $_{-7}$, and $_{-8}$) mGlus couple to $G_{i/o}$ G proteins and are often located presynaptically, where they modulate neurotransmitter release. The recent availability of selective pharmacological tools for mGlus has led to the identification of specific receptor subtypes as potential therapeutic targets for CNS disorders such as PD.

Several recent studies using group III mGlu-selective agonists, such as L-AP4 and ACPT-I, have demonstrated that these compounds have antiparkinsonian effects in rodent PD models, including reserpine-induced akinesia, haloperidol-induced catalepsy, and 6-hydroxydopamine lesion-induced motor deficits (Konieczny et al., 2007; Lopez et al., 2012, 2007, 2008; MacInnes et al., 2004; Sibille et al., 2007; Valenti et al., 2003). To determine the specific mGlu subtypes that mediate the antiparkinsonian effects of group III mGlu agonists, recent studies have taken advantage of subtype-selective agonists and positive allosteric modulators (PAMs) of $mGlu_4$ (East et al., 2010; Goudet et al., 2012; Jones et al., 2011a, 2011b; Marino et al., 2003; Niswender et al., 2008b). In rats, these compounds reverse reserpine-induced akinesia, haloperidol-induced catalepsy, and motor deficits caused by unilateral 6-hydroxydopamine lesion, suggesting that $mGlu_4$ activation is at least partially responsible for the antiparkinsonian effects of group III mGlu agonists in PD animal models. However, the potential contribution of other group III mGlu subtypes has not been fully elucidated. We now report that the $mGlu_8$ agonist (*S*)-3,4-dicarboxyphenylglycine (DCPG) (Thomas et al., 2001) has behavioral effects predictive of antiparkinsonian actions in several rodent models of PD when administered via an intracerebroventricular route of administration. Interestingly, reversal of akinetic motor deficits by DCPG requires a prolonged state of dopamine depletion or dopamine receptor blockade, suggesting that $mGlu_8$ function in the basal ganglia may differ in the intact versus dopamine-depleted states. These findings suggest that $mGlu_8$ activation may partially mediate the anti-akinetic effects of group III mGlu agonists in prolonged dopamine depletion models.

2. Materials and methods

2.1. Animals

Two hundred seventy-one third ventricle-cannulated (TVC) male Sprague–Dawley rats weighing 250–300 g were purchased from Taconic Farms, Inc. (Hudson, NY). Cannula placement (AP = −0.8 mm, ML = 0.0 mm and DV = −8.0 mm, relative to bregma) allowed infusion of non-brain penetrant drugs into the third ventricle. Cannula placement was visually verified following sacrifice for all animals used in these studies. Animals that underwent forelimb asymmetry testing were lesioned by unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle prior to TVC surgery. 6-OHDA lesions were functionally verified using an apomorphine-induced rotation test (performed by Taconic Farms, Inc.). On day 21 post-lesion, apomorphine (0.05 mg/kg, sc) was administered and rotations contralateral to the lesion were measured in a rotameter for 6 consecutive 5 min periods (30 min total). Only animals with greater than 180 rotations in 30 min or multiple 5 min periods of more than 6 rotations per minute were used for the forelimb asymmetry study. For studies that did not require intracerebroventricular (icv) drug administration, thirty-eight male Sprague–Dawley rats (250–300 g) that had not undergone TVC surgery were used (Harlan, Indianapolis, IN). Animals were maintained in accordance with the guidelines of the American Association for the Accreditation of Laboratory Animal Care under a 12 h light/dark cycle (lights on 06:00 to 18:00) with free access to food and water. All experiments were performed during the light cycle, were approved by Vanderbilt University's Institutional Animal Care and Use Committee, and conformed to guidelines established by the National Research Council *Guide for the Care and Use of Laboratory Animals*. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Drugs

(*S*)-3,4-DCPG and L-AP4 were purchased from Tocris Bioscience (Ellisville, MO). Haloperidol (free base) and reserpine were purchased from Sigma–Aldrich (St. Louis, MO). The decanoate salt of haloperidol was synthesized in-house. DCPG and L-AP4 were prepared in artificial cerebrospinal fluid (ACSF) and administered icv at a rate of 0.5–1 μl/min in the indicated volume. Injection cannulae were left in place for an additional 5 min after completion of infusion. Haloperidol (1.5 mg/kg) was dissolved in 0.2% lactic acid, and pH was adjusted to ~6.5 with 1 N NaOH. Haloperidol was administered intraperitoneally (ip) in a volume of 1 ml/kg. Reserpine (5 mg/kg, dissolved in 1% acetic acid) was prepared as described previously (Valenti et al., 2003), and administered subcutaneously (sc) in a volume of 1 ml/kg under light isoflurane anesthesia. Haloperidol decanoate (50–200 mg/kg) was dissolved in sesame oil using a mortar and pestle, and was administered intramuscularly (im) in a volume of 2 ml/kg; half of the dose was given in each femoral muscle. All drugs were prepared fresh on the day of the experiment.

2.3. Induction and measurement of catalepsy

For acute catalepsy studies, haloperidol (1.5 mg/kg, ip) was administered 2 h prior to baseline catalepsy measurement. For prolonged haloperidol-induced catalepsy studies, three doses of haloperidol (1.5 mg/kg, ip) were evenly spaced over 18–20 h prior to baseline catalepsy measurement. For haloperidol decanoate-induced catalepsy studies, animals were pretreated for the indicated time prior to measurement of catalepsy. Animals were returned to their home cages during the haloperidol pretreatment period. Catalepsy was assessed by placing each rat's forepaws on a horizontal bar positioned 6 cm above the testing surface and measuring the latency for the rat to remove one forepaw. Trials were ended after 60 s if no forepaw had been removed, and a score of 60 s was recorded for that trial. For reversal studies, rats were then given an icv infusion of either DCPG (2.5 or 10 nmol), L-AP4 (50 nmol or 100 nmol), or vehicle (ACSF). Rats were retested for catalepsy either 5 min after completion of drug infusion (haloperidol studies) or 10, 20, and 30 min after completion of infusion (haloperidol decanoate studies).

2.4. Induction and measurement of akinesia

Rats were treated with reserpine (5 mg/kg, sc) and returned to their home cages for either 2 h (acute treatment) or 18–20 h (prolonged treatment) prior to measurement of baseline akinesia. Locomotor activity was measured for 30 min by placing rats in photocell activity cages (Hamilton-Kinder, Poway, CA) equipped with 16 × 16 infrared beams. Akinetic rats then received an icv infusion of DCPG (2.5, 10, or 30 nmol), L-AP4 (50 nmol), or vehicle (ACSF), and activity was measured for an additional 30 min. For haloperidol decanoate characterization, locomotor activity was measured for 30 min at the indicated times after haloperidol decanoate administration following baseline catalepsy measurement as described above.

2.5. Measurement of striatal dopamine levels

Rats were treated with reserpine (5 mg/kg, sc) or vehicle for either 2 h (acute treatment) or 18–20 h (prolonged treatment) prior to sacrifice. Brains were removed rapidly and placed into ice-cold 0.9% NaCl solution. Tissue samples of caudate-putamen (CPu) were taken bilaterally, immediately weighed, and stored on dry ice. Samples were analyzed for striatal monoamine content using high performance liquid chromatography (HPLC) with electrochemical detection (Hackler et al., 2006). Briefly, samples were homogenized in 0.1 M trichloroacetic acid containing 10 mM sodium acetate, 100 μM EDTA, and 10.5% methanol (pH 3.8). Homogenized samples were centrifuged at 10,000 × g for 20 min, and the supernatant was removed and stored at −80 °C prior to measurement of dopamine content. Total protein concentration was determined by assaying the pellet using the BCA Protein Assay Kit (Pierce Chemical Company, Rockford, IL).

2.6. Estimation of D_2 receptor occupancy with PET imaging

D_2 dopamine receptor occupancy following haloperidol administration was measured using positron emission tomography (PET) as described previously (Jones et al., 2008; Tantawy et al., 2009). One week prior to the first day of testing, rats underwent surgery to install catheters in the jugular vein for radiotracer administration. On the first testing day, vehicle (0.2% lactic acid, ip) was administered 2 h prior to injection of ~13 MBq/0.2 mL [^{18}F]fallypride [(S)-N-[(1-allyl-2-pyrrolidinyl) methyl]-5-(3′-[^{18}F]fluoropropyl)-2,3-dimethoxybenzamide]. Rats were then returned to their home cages with free access to food and water. Sixty minutes after