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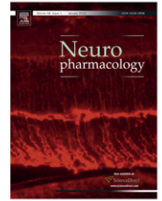

Metabotropic glutamate receptor 4 in the basal ganglia of parkinsonian monkeys: Ultrastructural localization and electrophysiological effects of activation in the striatopallidal complex

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ABSTRACT

Group III metabotropic glutamate receptors (mGluR4,7,8) are widely distributed in the basal ganglia. Injection of group III mGluR agonists into the striatopallidal complex alleviates parkinsonian symptoms in 6-hydroxydopamine-treated rats. *In vitro* rodent studies have suggested that this may be partly due to modulation of synaptic transmission at striatopallidal and corticostriatal synapses through mGluR4 activation. However, the *in vivo* electrophysiological effects of group III mGluRs activation upon basal ganglia neurons activity in nonhuman primates remain unknown. Thus, in order to examine the anatomical substrates and physiological effects of group III mGluRs activation upon striatal and pallidal neurons in monkeys, we used electron microscopy immunohistochemistry to localize mGluR4, combined with local administration of the group III mGluR agonist L-AP4, or the mGluR4 positive allosteric modulator VU0155041, to assess the effects of group III mGluR activation on the firing rate and pattern of striatal and pallidal neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated parkinsonian monkeys.

At the ultrastructural level, striatal mGluR4 immunoreactivity was localized in pre- (60%) and post-synaptic (30%) elements, while in the GPe, mGluR4 was mainly expressed pre-synaptically (90%). In the putamen, terminals expressing mGluR4 were evenly split between putative excitatory and inhibitory terminals, while in the GPe, most labeled terminals displayed the ultrastructural features of striatal-like inhibitory terminals, though putative excitatory boutons were also labeled. No significant difference was found between normal and parkinsonian monkeys. Extracellular recordings in awake MPTP-treated monkeys revealed that local microinjections of small volumes of L-AP4 resulted in increased firing rates in one half of striatal cells and one third of pallidal cells, while a significant number of neurons in both structures showed either opposite effects, or did not display any significant rate changes following L-AP4 application. VU0155041 administration had little effect on firing rates. Both compounds also had subtle effects on bursting and oscillatory properties, acting to increase the irregularity of firing. The occurrence of pauses in firing was reduced in the majority (80%) of GPe neurons after L-AP4 injection. Our findings indicate that glutamate can mediate multifarious physiological effects upon striatal and pallidal neurons through activation of pre-synaptic group III mGluRs at inhibitory and excitatory synapses in parkinsonian monkeys.

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1. Introduction

Drugs that antagonize glutamate transmission have been of interest for years as potential treatments for Parkinson's disease (PD), as they may reverse some of the major pathophysiologic hallmarks of the disease, specifically the increased glutamatergic corticostriatal and subthalamofugal transmission (Blandini et al., 1996; Chase et al., 2003; Greenamyre, 2001). However, early studies that focused on antagonizing ionotropic glutamate receptors as treatments for Parkinson's disease and other conditions were generally disappointing, largely due to the occurrence of debilitating side effects, which may have resulted from unwanted drug actions outside of the intended targets (Blandini and Greenamyre, 1998; Hughes, 1997; Smith et al., 2012). In contrast,

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because of their modulatory effects and more restricted regional distributions, the G-protein coupled metabotropic glutamate receptors (mGluRs) have become attractive targets for glutamate-based pharmacotherapies in Parkinson's disease (Breysse et al., 2003; Johnson et al., 2009; Lopez et al., 2007; Marino and Conn, 2006; Ossowska et al., 2007; Smith et al., 2012). These receptors are divided into three major groups based on their structure, pharmacology, and coupling to second messenger systems. Group I mGluRs (mGluR1 and mGluR5) are mainly expressed post-synaptically and increase neuronal excitability when activated, while group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) receptors are commonly found in pre-synaptic neuronal elements, where they decrease neurotransmitter release by inhibition of adenylyl cyclase.

In light of anatomical, electrophysiological and behavioral studies in rodents, it has become clear that activation of mGluR4 may represent a useful approach to normalize basal ganglia circuit activity, and to relieve PD motor symptoms (Beurrier et al., 2009; Johnson et al., 2009; Lopez et al., 2007, 2008; Marino et al., 2003; Smith et al., 2012; Valenti et al., 2003). In rats, mGluR4 is strongly expressed in the globus pallidus (GP), where it is localized predominantly in putative striatopallidal GABAergic terminals (Bradley et al., 1999; Corti et al., 2002). Activation of mGluR4 attenuates inhibitory post-synaptic currents induced by striatal stimulation in rat GP slices (Beurrier et al., 2009; Matsui and Kita, 2003; Valenti et al., 2003). It has been suggested that, through decreasing striatopallidal GABAergic transmission, mGluR4 activation may disinhibit GP neurons, thereby increasing the pallidal inhibitory drive to the abnormally overactive subthalamic nucleus (STN) and potentially correcting aberrant basal ganglia circuit activity in the parkinsonian state (Johnson et al., 2009; Marino and Conn, 2006; Niswender and Conn, 2010). Parkinsonian motor signs are, indeed, improved in rodent models of PD following intracerebral administration of group III mGluR agonists or mGluR4 positive allosteric modulators (Beurrier et al., 2009; Cuomo et al., 2009; Konieczny et al., 2007; Lopez et al., 2007; MacInnes et al., 2004; Marino et al., 2005, 2003; Valenti et al., 2003). The recent development of mGluR4 potentiators with reliable pharmacokinetic properties and efficient brain penetration has set the stage for trials of mGluR4-related PD pharmacotherapeutics (Engers et al., 2011; Niswender and Conn, 2010; Smith et al., 2012).

In light of the promising rodent data, we aimed to characterize the target sites and physiological effects of mGluR4-related drugs in the primate basal ganglia, using a combination of light and electron microscopic immunohistochemistry, local application of group III mGluR-related drugs in the striatopallidal complex, and *in vivo* single unit recordings of striatal and pallidal neurons in awake parkinsonian rhesus monkeys. Some of the findings of this study have been presented in abstract forms (Bogenpohl et al., 2010, 2011).

2. Material and methods

2.1. Animals

Eleven adult rhesus macaques (7 males, 4 females; 2–9 years old) were used in this study. All experiments were performed in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. Five of these monkeys were treated with weekly systemic injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 0.2–0.8 mg/kg/week; Sigma, St. Louis, MO; total cumulative doses ranged from 14 to 33 mg/kg, total treatment time ranged from 8 to 16 months) until moderate parkinsonian motor signs were observed.

The severity of parkinsonism was assessed as previously described (Kliem et al., 2010; Wichmann et al., 2001). Briefly, animals were transferred to an observation cage, in which locomotor behavior was measured by counting infrared beam breaks, and by direct visual quantification of the number of movements made by different body parts. A modified PD rating scale was also used to quantify parkinsonism. Following the development of moderate parkinsonian motor signs that remained stable for a period of at least 6–8 weeks after MPTP administration, two of the parkinsonian monkeys and three normal, untreated monkeys were trained to sit calmly in a restraint chair before being chronically implanted with transcranial recording chambers for electrophysiological experiments.

The remaining three MPTP-treated parkinsonian monkeys and three untreated animals were deeply anesthetized with pentobarbital (100 mg/kg i.v.) and transcardially perfused with cold oxygenated Ringer's solution, followed by a fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in a phosphate buffer (PB) solution, for immunohistochemistry. After perfusion, brains were removed from the skull, sliced coronally into thick (~1 cm) blocks, and post-fixed overnight in 4% paraformaldehyde. These blocks were cut into 60 μm-thick coronal sections using a vibrating microtome and stored at −20 °C in an anti-freeze solution, containing 30% ethylene glycol and 30% glycerol in PB, until ready for immunohistochemistry.

2.2. Immunohistochemistry

2.2.1. Pre-embedding immunoperoxidase for light microscopy

The localization of mGluR4 was achieved using a highly specific polyclonal antibody (Invitrogen, Carlsbad, CA; Catalog # 51-3100; 1:200 dilution) raised in rabbit against a 200 amino acid C-terminal fragment of the rat mGluR4 protein. This antibody is specific for the mGluR4a splice variant. Reactivity with other related proteins has not been detected on immunoblots of transfected cells expressing other mGluR subtypes (Invitrogen). This antibody does not stain brain tissue from mGluR4 knockout mice (unpublished data). Depletion of the dopaminergic nigrostriatal system in MPTP-treated animals was confirmed in 3 of the 5 animals in this study (the 2 remaining animals are still alive at the time of this report) by staining sections at the level of the striatum and the substantia nigra with mouse anti-tyrosine hydroxylase (TH) antibodies (1:1000, Millipore; not shown).

Brain sections taken from various anteroposterior levels of the basal ganglia (see also below) in 2 normal and 2 MPTP-treated monkeys were processed for light microscopy (LM) immunohistochemical localization of mGluR4. Prior to immunohistochemical (IHC) processing, brain sections were washed with phosphate buffered saline (PBS; 0.01 M, pH 7.4), treated with a 1% sodium borohydride solution for 20 min, and washed in PBS once more.

Sections were incubated for 1 h in PBS containing 10% normal goat serum (NGS), 1% bovine serum albumin (BSA), and 0.3% Triton X-100, followed by incubation in the primary antibody solution containing 1% NGS, 1% BSA, and 0.3% Triton X-100 in PBS for 48 h at 4 °C. Sections were then rinsed three times in PBS and incubated in the secondary antibody solution containing 1% NGS, 1% BSA, 0.3% Triton X-100, and biotinylated goat anti-rabbit IgGs (Vector Laboratories, Burlingame, CA) at a dilution of 1:200 for 90 min at room temperature. After three rinses in PBS, sections were incubated for 90 min in avidin-biotin peroxidase complex (ABC) solution at a dilution of 1:100 (Vectastain standard ABC kit, Vector Laboratories) including 1% BSA.

To reveal the antigenic sites, sections were first rinsed with PBS and Tris buffer (50 mM; pH 7.6), then incubated in a solution containing 0.025% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma), 10 mM imidazole, and 0.005% hydrogen peroxide in Tris buffer for 10 min. Sections were then washed several times in PBS, mounted on gelatin-coated glass slides, dehydrated, and coverslipped with Permount™.

The slides were scanned at 20× using a ScanScope CS scanning light microscope system (Aperio Technologies, Vista, CA). Digital representations of the slides were saved and analyzed using ImageScope software (Aperio Technologies).

2.2.2. Pre-embedding immunoperoxidase for electron microscopy

Sections containing the striatopallidal complex from 3 normal and 3 MPTP-treated monkeys were transferred to a cryoprotectant solution containing 25% sucrose and 10% glycerol in PB (0.05 M, pH 7.4) for 20 min and then placed in a −80 °C freezer for 20 min to permeabilize cell membranes. They were then thawed through washes in decreasing concentrations of cryoprotectant solution until being washed in PBS. The subsequent tissue processing was identical to that used for light microscopy, up to the point of DAB revelation, with two important differences: Triton X-100 was omitted from all solutions, and sections were incubated in the primary antibody solution for 48 h at 4 °C.

After DAB revelation, the tissue was rinsed in PB (0.1 M, pH 7.4) and treated with 1% osmium tetroxide for 20 min. It was then rinsed with PB and dehydrated with increasing concentrations of ethanol, up to 100%. Uranyl acetate (1%) was added to the 70% EtOH dehydration solution and incubated for 35 min in order to increase the contrast of membranes in the electron microscope. After alcohol dehydration, sections were treated with propylene oxide, embedded in epoxy resin (Durcupan ACM; Fluka, Buchs, Switzerland) for at least 12 h, mounted onto slides, and placed in a 60 °C oven for 48 h to cure the resin.

2.2.3. EM observations and analysis

Small blocks of tissue from the dorsolateral putamen or GPe (12–15 mm anterior to the interaural coronal plane; Paxinos et al., 2000) were cut out from

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