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Metabotropic glutamate receptor type 5 in levodopa-induced motor complications

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Abstract

Metabotropic glutamate receptors type 5 (mGluR5) are implicated in regulation of synaptic plasticity and learning, and were the focus of our investigation in human Parkinson's disease (PD) patients with dyskinesias and wearing-off, and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkeys with dyskinesias. Using the selective mGluR5 ligand [^{3}H]ABP688 autoradiography, we measured mGluR5 in brain slices from 11 normal and 14 PD patients and from MPTP monkeys, in relation to motor complications (dyskinesias and wearing-off) associated with treatment with L-dopa. In 16 monkeys with a bilateral MPTP lesion and four controls, [^{3}H]ABP688 specific binding was elevated in the striatum of dyskinetic L-dopa-treated MPTP monkeys but not in MPTP monkeys without dyskinesias compared to controls. PD patients with motor complications (either dyskinesias or wearing-off) had higher [^{3}H]ABP688 specific binding compared to those without motor complications and controls in putamen, external and internal globus pallidus. Elevated glutamatergic transmission as measured with increased mGluR5 specific binding was associated with motor complications and its antagonism could be targeted for their treatment.

Keywords: Glutamate receptor; mGluR5; Human; MPTP monkey; Wearing-off; Dyskinesias

1. Introduction

Glutamate, the brain major excitatory neurotransmitter, acts through three ionotropic (NMDA, AMPA and kainate) and eight metabotropic receptors (mGluR1–8) (Ferraguti and Shigemoto, 2006). Glutamate excess release in the basal ganglia motor circuit plays a critical role in expression of Parkinson's disease (PD) symptoms and L-dopa-induced motor complications (Calon et al., 2003a; Gubellini et al., 2006). Motor complications are common in PD patients and develop in 38–50% of them within 2 years and 50–80% after 5–10 years of therapy (Stacy et al., 2006). Moreover, it was reported that wearing-off is the first type of motor fluctuations to develop within 5–6 months of therapy (Fahn et al., 2004). Dyskinesias belong to motor adverse effects linked to replacement by L-dopa of dopamine (DA) lost in the striatum due to nigrostriatal DA neurons degeneration in PD (Stacy et al., 2006). Amantadine was the first glutamatergic drug shown to reduce severity of dyskinesia without worsening parkinsonian symptoms (Stocchi et al., 2008). Ionotropic glutamate receptor antagonists have been tested for their antidyskinetic activity in PD patients (Chase

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and Oh, 2000) and animal models such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey (Hadj Tahar et al., 2004). But the utility of these drugs has stumbled over significant adverse effects such as cognitive impairment in many patients (Stocchi et al., 2008). Attention has now turned to metabotropic glutamate receptors proposed to be activated mainly when there is excess glutamate in the synaptic cleft and activate perisynaptic receptors thus acting when glutamate transmission is enhanced (Konradi et al., 2004). mGluR5 have a discrete brain distribution (Hintermann et al., 2007) and an antagonistic interaction between mGluR5 and D2 DA receptors is described (Fuxe et al., 2008). This functional specificity in addition to their high striatal levels makes this receptor an interesting pharmacological target (Conn et al., 2005). Pharmacological antagonism of mGluR5 was shown to inhibit expression of dyskinesias in a rodent model of PD (Mela et al., 2007).

In agreement with results on mGluR5 labeled with [^{3}H]MPEP in MPTP monkeys (Samadi et al., 2008), the present study, using [^{3}H]ABP688 a novel high affinity and selective mGluR5 antagonist (Hintermann et al., 2007), shows elevated basal ganglia mGluR5 specific binding in PD patients related to their motor complications and MPTP monkeys with L-dopa-induced dyskinesias (LID).

2. Materials and methods

2.1. Animals and treatments

The experiments were performed in accordance with standards of the Canadian Council on Animal Care using 24 ovariectomized female *Macaca fascicularis* monkeys weighing 2.8–6.5 kg.

The first experiment included four monkeys rendered hemiparkinsonian by an unilateral intranigral infusion of 3 mg of the neurotoxin MPTP (Sigma–Aldrich Canada, Oakville, Ontario) as we described (Morissette and Di Paolo, 2009).

The second experiment comprised 20 monkeys including four drug-naïve monkeys used as normal controls and 16 MPTP monkeys divided in four groups ($n = 4$ in each group). One group was injected with saline and served as untreated MPTP monkeys. Three other groups were treated for 4 weeks with 100 mg L-dopa and 25 mg benserazide (Prolopa®: Hoffmann-La Roche, Mississauga, Ontario) (termed "L-dopa" thereafter), L-dopa + CI-1041 (10 mg/kg) (Hadj Tahar et al., 2004) or L-dopa + cabergoline (a dose from 0.015 to 0.035 mg/kg) (Belanger et al., 2003). CI-1041 is a selective NR1A/2B receptor antagonist and cabergoline a long acting dopamine D2 receptor agonist. The detailed behavioral evaluation of these monkeys was previously reported (Belanger et al., 2003; Hadj Tahar et al., 2004). The different factors which might influence the development of dyskinesias (i.e., total dose of MPTP, time after MPTP exposure and start of chronic treatment, basal parkinsonian score) were similar for all groups (Belanger et al., 2003; Hadj Tahar et al., 2004). The spontaneous behavior of the monkeys during the total effect of a given drug was assessed through a one-way screen. Antiparkinsonian effects of L-dopa were similar in all monkeys. All four animals treated with L-dopa developed dyskinesias, while CI-1041 and cabergoline completely prevented the induction of dyskinesias in three monkeys in each group. Only one monkey in the two latter groups developed mild dyskinesias (Belanger et al., 2003; Hadj Tahar et al., 2004).

At the end of experiments, monkeys were euthanized by an over dose of sodium pentobarbital. The time interval between last drug treatments and euthanasia was 24 h. Brains were removed and placed in isopentane for less than 30 s (−40 °C), then kept frozen at −80 °C until used. Brains were cut into coronal sections of 12 μm on a cryostat (−18 °C).

In the first experiment, samples of anterior (level ac + 3 mm) and posterior (ac − 2 mm) striatum were prepared according to the atlas of Martin and Bowden (2000).

In the second experiment, hemisected brains were cut to sample anterior striatum (levels A18–A22) and posterior striatum and globus pallidus (levels A15–A18) according to the atlas of Szabo and Cowan (1984).

2.2. Patients

Samples from PD patients included in this study were selected from a large prospective study on L-dopa-induced motor complications, evaluated by the same neurologist (AHR) at 6–12 months interval (Rajput et al., 2002) and were previously investigated (Calon et al., 2002, 2003a,b, 2004). The clinical profiles of patients and motor complications (LID and wearing-off) were entered prospectively after each clinical assessment of the patients (Rajput et al., 2002). LID were defined as biphasic or peak-dose choreic/dystonic abnormal involuntary movements; wearing-off as predictable decline in motor benefit at the end of the dose in a patient with a previously stable response (Rajput et al., 2002). Patients were divided into groups according to development of motor complications (Calon et al., 2002, 2003a,b, 2004).

2.3. Autopsy and handling of brain material

Autopsies of all human subjects were done within 24 h of death and PD diagnosis confirmed based on marked neuronal loss in substantia nigra *pars compacta*, presence of Lewy body, and absence of other pathological changes that may account for parkinsonian symptoms (Rajput et al., 2002). Brains were processed as previously described (Calon et al., 2002, 2003a,b, 2004).

2.4. Tissue preparation and measures of extent of dopamine denervation

Small punches of human cerebral cortex were used to determine tissue pH, to assess its preservation (Calon et al., 2003a). Measures of extent of denervation included assay of