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Effect of the metabotropic glutamate receptor type 5 antagonists MPEP and MTEP in parkinsonian monkeys

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ABSTRACT

Brain glutamate overactivity is well documented in Parkinson's disease (PD) and antiglutamatergic drugs have been proposed to relieve PD symptoms and decrease dyskinesias. Metabotropic glutamate receptors are topics of recent interest in PD. This study investigated the effects of the metabotropic glutamate receptors type 5 (mGluR5) antagonists MPEP and MTEP on motor behavior in monkeys with a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion to model PD and treated with L-Dopa the gold standard therapy. Six *Macaca fascicularis* MPTP monkeys were initially treated repeatedly with L-Dopa; this treatment increased their locomotion and reduced their parkinsonian scores but also induced dyskinesias. Then, a dose–response of MPEP and MTEP (1.5–30 mg/kg) administered 15 and 30 min respectively prior to L-Dopa, showed that the antiparkinsonian activity of L-Dopa was generally maintained as measured with locomotion and antiparkinsonian scores as well as the onset and duration of the L-Dopa response. Interestingly the mean dyskinesia score during all the duration of the L-Dopa motor effect, the 1 h peak period dyskinesias scores as well as the maximal dyskinesias scores were dose-dependently reduced with both drugs reaching statistical significance at 10 and 30 mg/kg. Our results showed a beneficial antidyskinetic effect of blocking mGluR5 in L-Dopa-treated MPTP monkeys. This supports the therapeutic use of an mGluR5 antagonist to restore normal brain glutamate neurotransmission in PD and decrease dyskinesias.

1. Introduction

The most effective and commonly used treatment for Parkinson disease (PD) is to restore the dopamine (DA) loss with its precursor levodopa (L-Dopa). However, in the long term approximately 80% of L-Dopa treated patients will develop abnormal involuntary movements including L-Dopa-induced dyskinesias (LID) (Stacy et al., 2006; Van Gerpen et al., 2006). Excessive glutamate activity in the basal ganglia plays a critical role in expression of PD symptoms and L-Dopa-induced motor complications (Calon et al., 2003; Gubellini et al., 2006) and the inhibition of its action can alleviate dyskinesias (Chase and Oh, 2000). Antagonists of the ionotropic glutamate receptors have shown antidyskinetic activity in PD patients (Verhagen Metman et al., 1998) and animal models such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey (Hadj Tahar et al., 2004). However, these drugs have significant adverse effects such as cognitive impairment in many patients (Stocchi et al., 2008) which significantly limit their use.

The action of glutamate is also mediated through metabotropic glutamate receptors (Meldrum, 2000) a family of G-protein coupled receptors comprising eight subtypes. One subtype, the metabotropic glutamate type 5 (mGluR5) is highly expressed in the striatum. An antagonistic interaction between mGluR5 and D2 DA receptors (Fuxe et al., 2008) as well as a mGlu5 receptor mediated positive modulatory action on the NMDA receptor responses (Pisani et al., 2001) has been described making this receptor an interesting pharmacological target (Conn et al., 2005). Over the past three years, pharmacological antagonism of mGluR5 with the selective antagonists 2-methyl-6-(phenylethynyl)pyridine (MPEP) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) was shown to inhibit expression of dyskinesias in the 6-hydroxydopamine (6-OHDA) lesioned rat model of PD (Gravius et al., 2008;

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Levandis et al., 2008; Mela et al., 2007; Rylander et al., 2009; Yamamoto and Soghomonian, 2009). However, no article is yet published in other PD models including in primate PD models to support the relevance of these interesting rat findings for humans although one group has recently reported in an abstract form the effect of MTEP in monkeys (Johnston et al., 2009a,b). In addition, mGluR5 specific binding was shown to be increased in the basal ganglia of parkinsonian monkeys that developed dyskinesias following chronic L-Dopa treatment (Samadi et al., 2008). Taken together, these studies suggested that antagonism of mGlu5 receptor may be useful in the treatment of L-Dopa-induced dyskinesias in PD. Therefore, the present study investigated the effect of the pharmacological blockade of mGluR5 with MPEP and MTEP on L-Dopa-induced dyskinesia in MPTP monkeys.

2. Materials and methods

2.1. Animals

Six female ovariectomized cynomolgus monkeys (*Macaca fascicularis*) weighing between 2.8 and 4.4 kg were used for these experiments. Handling of the primates was performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All procedures, including means to minimize discomfort, were reviewed and approved by the Institutional Animal Care Committee of Laval University. The animals were rendered parkinsonian by continuous infusion of MPTP (Sigma–Aldrich, Canada, Oakville, Ontario) using subcutaneous osmotic minipumps (Alzet, 0.5 mg/24 h) until they developed a stable parkinsonian syndrome. After 1–3 months of stabilization, animals were treated daily with L-Dopa 100/25 capsule p.o. (Prolopa, Hoffmann–La Roche; a mixture of 100 mg of L-Dopa and 25 mg benserazide) for a few months until clear and reproducible dyskinesias developed. Thereafter, animals received repeated administrations 3 times per week with L-Dopa capsules p.o. (Prolopa 100/25 or 50/12.5 depending of the animal) to maintain their priming and comfort.

2.2. Drugs

MPEP (Novartis, Basel, Switzerland; Tocris, USA, Ellisville, Missouri) and MTEP (Axxora, USA, San Diego, California) were prepared on every experimental day using a 5% (v/v) Tween 80, 0.1% (v/v) ethanol aqueous solution. MPEP and MTEP were first solubilized in a small amount of ethanol 100% and then 100 ml of 5% (v/v) Tween 80 aqueous solution was added. MPEP was totally soluble at all doses tested. Higher doses of MTEP were not completely soluble (10 and 30 mg/kg) and were mixed thoroughly with a high speed homogenizer (Polytron, Brinkman Instruments, Westbury) for 15 min and then kept under gentle stirring until administration. A volume between 12 and 20 ml of MPEP and MTEP suspensions were administered by nasogastric gavage. L-Dopa methyl ester and benserazide (Sigma–Aldrich Canada, Oakville, ON) were dissolved in sterile 0.9% saline solution and pH adjusted to 7.

2.3. Behavioral MPEP and MTEP acute study

The MPEP or MTEP tests were always 2 days following the last L-Dopa capsule administration. The animals were first evaluated following vehicle (Tween 80 5%, ethanol 0.1%) administration alone (vehicle control) and with vehicle + L-Dopa/benserazide s.c. (called thereafter L-Dopa). Behavioral measures after L-Dopa/benserazide were done three times and repeated twice; these measures were not different and their mean is presented. L-Dopa doses were adjusted for each monkey and varied between 15 and 35 mg/kg s.c. and were always given simultaneous with a fixed dose of benserazide (50 mg total). Four doses (1.5, 3, 10, 30 mg/kg) of MPEP or MTEP with L-Dopa were tested in ascending doses order (vehicle, L-Dopa (3 times), MPEP 1.5 mg/kg + L-Dopa, MPEP 3.0 mg/kg + L-Dopa, MTEP 1.5 mg/kg + L-Dopa, MTEP 3.0 mg/kg + L-Dopa then a month later: L-Dopa (3 times), MTEP 10 mg/kg + L-Dopa, MTEP 30 mg/kg + L-Dopa, MPEP 30 mg/kg + L-Dopa) except MPEP 10 mg/kg + L-Dopa that was tested last for scheduling reasons. MPEP and MTEP were given 15 and 30 min respectively before L-Dopa administration. At least four days of washout were left between each MPEP or MTEP experiments.

The animals were observed through a one-way screen and were scored "live" for antiparkinsonian and dyskinetic responses for all the duration of the L-Dopa response.

2.3.1. Parkinsonian score

A disability scale developed in our laboratory was used to evaluate the parkinsonian syndrome in MPTP monkeys (Hadj Tahar et al., 2004; Samadi et al., 2003). Briefly, behaviors were scored every 15 min (maximal score: 16): a) Posture: normal = 0, flexed intermittent = 1, flexed constant = 2, crouched = 3; b) Mobility: normal = 0, mild reduction = 1, moderate reduction = 2, severe reduction = 3; c) Climbing: present = 0, absent = 1; d) Gait: normal = 0, slow = 1, very slow = 2, very slow with freezing = 3; e) Grooming: present = 0, absent = 1; f) Vocalization: present = 0, absent = 1; g) Social interaction: present = 0, absent = 1; h) Tremor: absent = 0, mild action tremor = 1, moderate action tremor = 2, resting tremor = 3.

2.3.2. Dyskinetic response

Dyskinesias were scored every 15 min for all the duration of the L-Dopa effect (Hadj Tahar et al., 2004; Samadi et al., 2003). Dyskinesias were rated for the face, neck, trunk, arms and legs as follows: None = 0; Mild (occasional) = 1; Moderate (intermittent) = 2; Severe (continuous) = 3. The dyskinetic score obtained was the sum of the scores for all body segments (maximal score: 21).

2.3.3. Locomotor response

Locomotor activity was monitored continuously with an electronic motility monitoring system fixed on each cage (Datascience, St. Paul, Minnesota, USA). Computerized mobility counts were obtained every 5 min.

2.4. Data analysis

For each treatment day and for each monkey, a mean parkinsonian score and a mean dyskinetic score (total period) were obtained by averaging all 15 min scores obtained for the duration of the response. Moreover, for dyskinesia, values for the 1 h peak period and the maximum dyskinesia score were computed. Parkinsonian and dyskinesia scores were analyzed with a Friedman non-parametric test followed by a multiple comparisons test based on rank. In figures, parkinsonian and dyskinesia scores are illustrated as follows: median (horizontal line), interquartile range (box) and range (bars). Values for locomotor activity, duration and delay of responses (in minutes) were analyzed by an ANOVA for repeated measures followed by a Dunnett's multiple comparisons test. Mean ± S.E.M. are represented in the graphs illustrating the results of locomotor activity, duration and delay of L-Dopa response. A $p < 0.05$ was considered significant.

3. Results

L-Dopa administered alone to MPTP monkeys induced a decrease (improvement) of parkinsonian scores; this was maintained with the addition of MPEP at all doses tested (Fig. 1A). Similar decreases (improvement) of parkinsonian scores were obtained with L-Dopa + MTEP, but at the highest doses of 30 mg/kg of MTEP, the mean parkinsonian scores were not significantly reduced. L-Dopa administered alone to MPTP monkeys increased their locomotor activity and this was not changed with the addition of MPEP or MTEP at all doses tested (Fig. 1B). Addition of MPEP to L-Dopa did not delay the onset of the L-Dopa antiparkinsonian effect whereas treatment with 10 mg/kg of MTEP delayed the onset of the L-Dopa effect (22.2 ± 1.7 min, $p < 0.05$ vs L-Dopa alone 16.2 ± 0.9 min, 37.0%) (Fig. 1C). The duration of the antiparkinsonian effect of L-Dopa alone (179 ± 9 min) was increased slightly (9%) at 3 and 10 mg/kg MPEP tested (196 ± 10 min and 195 ± 11 min respectively, $p < 0.05$ vs L-Dopa alone) while the duration of the L-Dopa effect was decreased with MTEP only at 30 mg/kg (153 ± 18 min, $p < 0.05$ vs L-Dopa alone, 15%) (Fig. 1D). No abnormal behavior was observed at any of the MPEP or MTEP doses tested.

L-Dopa-induced choreic and dystonic dyskinesias in these MPTP monkeys that were dose-dependently reduced with the addition of MPEP and MTEP. The dyskinesias scores during the total period of the L-Dopa motor effect were decreased by 50% and 22% with the addition of MPEP 10 and 30 mg/kg and by 41% and 48% with MTEP 10 and 30 mg/kg respectively (Fig. 2A). Dyskinesias scores during the 1 h peak period were also decreased by 39% and 29% with MPEP 10 and 30 mg/kg and by 44% and 29% with MTEP 10 and 30 mg/kg respectively (Fig. 2B). The maximum dyskinetic scores of the MPTP monkeys were significantly reduced only with MPEP 10 mg/kg (decreased by 23%) and at the two highest doses tested of MTEP 10 and 30 mg/kg (decreased by 33 and 21% respectively) (Fig. 2C).

4. Discussion

Involvement of an excessive glutamate transmission in the basal ganglia has been proposed in the pathophysiology of PD and