L-DOPA-Induced Motor Impairment and Overexpression of Corticostriatal Synaptic Components Are Improved by the mGluR5 Antagonist MPEP in 6-OHDA-Lesioned Rats

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Abstract

Levodopa (L-DOPA) is still the most effective drug for the treatment of Parkinson's disease (PD). However, the long-term therapy often triggers L-DOPA-induced dyskinesia (LID). Metabotropic glutamate receptor type 5 (mGluR5) is abundant in the basal ganglia, and its inhibition is thought to modulate postsynaptic excitatory synaptic transmission and glutamate hyperactivity in PD and LID. In this report, we examined the effects of mGluR5-specific antagonist 2-methyl-6-(phenyl-ethynyl)pyridine (MPEP) on LID and synaptic components in the PD model rat. We found the selective mGluR5 antagonist MPEP attenuated abnormal involuntary movements, prolonged the duration of rotational response, reversed the decrease of left forepaw adjusting steps, and reduced overexpression of striatal mGluR5 in the LID rats. Moreover, our results showed much thicker postsynaptic densities, narrower synapse cleft, as well as the increased ratio of perforated synapses induced by L-DOPA treatment, while coadministration of L-DOPA and MPEP reversed these postsynaptic effects. Finally, MPEP reduced overexpression of the two postsynaptic proteins (PSD-95 and SAP102) induced by L-DOPA treatment. Hence, these results provide evidence that aberrant neural plasticity at corticostriatal synapses in the striatum is closely correlated with the occurrence of LID, and targeted inhibition of mGluR5 by MPEP alleviates LID in the PD rat model.

Keywords

Parkinson's disease, L-DOPA-induced dyskinesia, mGluR5, MPEP, postsynaptic densities

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Introduction

Parkinson's disease (PD), one of most common neurodegenerative diseases, is characterized by progressive loss of dopaminergic neurons at the substantia nigra pars compacta, resulting in remarkably decreased dopamine levels in the striatum (Bastide et al., 2015). Although Levodopa (L-DOPA) replacement is still the most effective therapy for PD, its long-term administration often causes motor complications, such as motor fluctuations and abnormal involuntary movements (AIMs), namely, L-DOPA-induced dyskinesia (LID). These complications are often difficult to deal with and greatly influence PD patients' quality of life (Fabbrini et al., 2007). Following 2 years of L-DOPA replacement, about 30%

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of patients initially developed LID, and then the percentage increased during treatment, up to 95% 15 years after treatment (Ahlskog and Muenter, 2001; Hely et al., 2005).

The underlying mechanisms for LID are still elusive. Previous studies have shown that LID is associated with aberrant synaptic plasticity in the striatum that could be attributed to an imbalance between glutamate and dopamine signaling (Cenci and Lundblad, 2006; Jenner, 2008; Blandini and Armentero, 2012; da Silva et al., 2018; C. Liu et al., 2018). The structural or functional changes of synapses are critical for the alteration of synaptic plasticity and thus are vital for the development of PD and LID. Therefore, targeting glutamate receptors might be a therapeutic strategy for LID symptoms. Clinical investigations showed that amantadine, a noncompetitive N-methyl-D-aspartate receptor antagonist, is the one only drug in clinical practice that might reduce the severity of LID in a proportion of PD patients without exacerbating parkinsonian symptoms (Verhagen Metman et al., 1998; Sawada et al., 2010; Meissner et al., 2011). However, its therapeutic effect for dyskinesia is yet temporary. Notably, this drug causes cognitive impairment at high doses (Stocchi et al., 2008). Apart from ionotropic glutamate receptors, much attention turns to metabotropic glutamate receptors, especially metabotropic glutamate receptor type 5 (mGluR5) at postsynaptic sites, which are enriched in the structures of basal ganglia. It is thought that the proper inhibition of mGluR5 modulates postsynaptic excitatory synaptic transmission and glutamate-related hyperactivity in PD and LID (Conn et al., 2005; Sebastianutto and Cenci, 2018).

Prior studies in parkinsonian patients and animal models suggested that blockade of mGluR5 signaling might be regarded as a potential therapeutic strategy for PD and LID (Masilamoni and Smith, 2018). Previous studies found that mGluR5 specific binding was upregulated in the basal ganglia of parkinsonian patients with motor complications and parkinsonian monkeys with LID (Samadi et al., 2008; Ouattara et al., 2010, 2011; Morin et al., 2013a, 2013c, 2014, 2016). In the 6-hydroxydopamine (6-OHDA)-lesioned rat model, 2-methyl-6-(phenylethynyl)pyridine (MPEP) and [3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine] (MTEP), two specific mGluR5 antagonists, alleviated LID (Dekundy et al., 2006; Mela et al., 2007; Gravius et al., 2008; Levandis et al., 2008). In 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys, a few of mGluR5 antagonists, such as MPEP, MTEP, and AFQ056, were observed to inhibit LID (Johnston et al., 2010; Morin et al., 2010; Rylander et al., 2010; Gregoire et al., 2011; Rascol et al., 2014) and reduced the occurrence of motor complications induced by L-DOPA following 1 month administration (Morin et al., 2013a). Moreover, two other antagonists for mGluR5 AFQ056

and ADX-48621 were reported to attenuate LID in PD patients with a better tolerance and no exacerbation of motor symptoms (Addex Therapeutics, Geneva, Switzerland (Media Release), 2012; Stocchi et al., 2013; Rascol et al., 2014). However, the underlying mechanisms of the antidyskinetic activity of mGluR5 antagonists remain unknown. In this study, we speculated that the therapeutic effects might be associated with the regulation of aberrant synaptic activity.

The postsynaptic density (PSD) localized at the excitatory synapses is a postsynaptic active zone consisting of various membrane-associated guanylate kinases (MAGUKs), including PSD-95, PSD-93, SAP102, and SAP97 (Chen et al., 2011; Sheng and Kim, 2011). PSD-95, the major scaffolding component, plays a critical role in synaptic strength. Moreover, it locates at the junction of regulation of glutamate and dopamine signaling (Yao et al., 2004; Porras et al., 2012), especially as glutamatergic and dopamine axons form a synaptic complexes at postsynaptic dendritic spines in the striatum (Kim and Sheng, 2004). Interestingly, the expression of PSD-95 was greatly increased in the 6-OHDA-lesioned side in PD model rats treated by L-DOPA (Nash et al., 2005). Nevertheless, the mechanisms for expression of PSD-95 in LID remain unclear. SAP102, as one scaffolding protein member of PSD-95-like MAGUKs, plays essential roles in synaptic organization and plasticity, especially in synapse development and synaptogenesis (Murata and Constantine-Paton, 2013). However, there is no evidence to show the role of SAP102 in LID.

This study aimed to investigate the effects of mGluR5-specific antagonist MPEP on LID and synaptic activity at the striatum of 6-OHDA-lesioned hemi-parkinsonian animals and to examine the involvement of striatal mGluR5 expression in the LID.

Materials and Methods

Animals

Male adult Sprague–Dawley animals (weighing 180-220g) were obtained from the Center for Experimental Animals, Soochow University, China (certificate No. 20020008, Grade II). Animals were maintained under the following conditions: 12h:12h light/dark cycle, 22°C–23°C, as well as food and water available ad libitum. The animals were housed in the facilities for 1 week before the experiments began. All procedures on the utilization of laboratory animals followed Animal Care and Use Guidelines in China. The number of animals and their sufferings were minimized. This study was approved by the ethics committee of the Second Affiliated Hospital of Soochow University.

Reagents

6-OHDA, ascorbic acid, L-DOPA methyl ester, benserazide, and MPEP were purchased from Sigma-Aldrich (St Louis, MO). Apoamorphine was purchased from Tocris (Bristol, UK). Rabbit monoclonal antibody against the mGluR5 receptor was obtained from Abcam (New Territories, Hong Kong, China), Rabbit monoclonal antibody against the PSD-95 receptor was obtained from Cell Signaling Technology (USA), anti-SAP102 was purchased from NeuroMab (Antibodies, Co., Davis, CA), anti-β-actin antibody was purchased from Beyotime Biotechnology (Shanghai, China).

6-OHDA Lesion and Screening of Rotational Responses

To establish 6-OHDA-induced parkinsonian model, the animals were anesthetized with chloral hydrate (3.6%, 0.18 g/kg i.p.) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Based on an adult rat brain atlas (Paxinos and Watson, 1986), 8 μg of 6-OHDA (dissolved in a volume of 4 μl saline containing 0.02% ascorbic acid) was injected unilaterally into the right medial forebrain bundle (leading to nigrostriatal lesion) with a 10-μl Hamilton syringe at the two following coordinates: (a) anteroposterior (AP), −1.8 mm; left (L) −2.5 mm; and dorsoventral (DV), −8.0 mm from Bregma. (b) AP, −1.8 mm; L, −2.5 mm; and DV, −7.5 mm from Bregma. A total of 16 μg 6-OHDA were injected at the two coordinates (8 μl for each site). 6-OHDA was freshly prepared in the dark to prevent autoxidation and delivered at a rate of 0.5 μl/min by a 10-μl microinjector. Before the slow withdrawal, the syringe was retained for about 5 min to allow for diffusion of the toxin and to avoid toxin reflux. After 2 weeks, the lesioned animals were delivered with apomorphine at a dose of 0.25 mg/kg (dissolved in physiological saline, i.p.) and were kept in a 40-cm-diameter round kettle. The numbers of contralateral rotations of each rat were recorded visually within 30 min; the animals with over seven contralateral turns within 1 min were considered as parkinsonian rats and chosen for further experiments.

The Design of Experiments

Two weeks following apomorphine detection, successfully induced PD-like animals were randomly classified into four groups (n = 10 for each group): (a) intraperitoneal (i.p.) injection with saline twice daily (the control group); (b) administration of L-DOPA (25 mg/kg L-DOPA plus 6.25 mg/kg benserazide in saline, the L-DOPA group) twice daily; (c) i.p. injection of MPEP (1.5 mg/kg in saline, the MPEP group) twice daily; and (d) i.p. injection of MPEP (1.5 mg/kg in saline) twice daily 30 min after an injection of 25 mg/kg L-DOPA plus 6.25 mg/kg benserazide twice daily (the coadministration group). The four groups were all administered for 21 days. The dose of L-DOPA was used based on our previous study (Huang et al., 2011), while the dose for MPEP was selected according to another study (Levandis et al., 2008).

Behavioral Tests

The record of AIMs triggered by L-DOPA was visually collected during behavioral tests (Lundblad et al., 2002) and divided into three kinds: (a) axial AIM: dystonic posture of the upper part of the body toward the un-lesioned side, (b) limb AIM: movements from the forelimb and the paw toward the un-lesion side, and (c) orolingual AIM: jaw movements and tongue protrusion. Each kind was scaled based on a severity ranged from 0 to 4: 0 indicates *lack of AIM*, 1 indicates *existence during less than half 1 min*, 2 indicates *existence during over half 1 min*, 3 indicates *persistent existence but inhibited by external stimuli*, and 4 indicates *persistent existence without the inhibitory possibility*. Theoretically, the maximal score achieved by one rat in a session was 144 (the maximum score for one monitoring period was 16 and the number of monitoring periods for one session was 9). Each AIM was quantified within 1 min and present as time which the rat stayed for each movement.

Following the treatment of L-DOPA, rotational behavior was assessed instantly, suggesting a motor impairment markedly triggered by L-DOPA in PD models (Lundblad et al., 2003). The duration of rotational responses was viewed as the time between the first 5-min interval as turning was greater than 20% of the peak value and the first interval as turning was lower than 20% of the peak value. The peak number of contralateral turns in any 5-min interval was viewed as the peak value of rotation.

To investigate the change in forelimb akinesia under different conditions, animals were subjected to the stepping test (Olsson et al., 1995). Briefly, at 30 min following L-DOPA injection, animals were moved across a table at a speed of 90 cm/5 s. Animals were caught at the rear part of the trunk by one hand of the researcher with their rear limbs upright and the ipsilateral forepaw was firmly grasped by another hand to make weight on the contralateral forepaw. The test consisted of five trials and the average score of five trials was used for analysis.

The behavioral evaluation of AIM, rotational response duration, and forepaw adjusting steps were performed on the 2, 9, 11, 18, and 21 days after L-DOPA application.

Western Blotting

Animals were anesthetized with 3.6% chloral hydrate (0.18 g/kg, i.p.) for 30 min after the last administration,

rats were sacrificed and their brains were quickly taken out. Then the lesioned striatum was separated and stored at −70°C for Western blots. The tissues from the dorsal striatum were homogenized and centrifuged at 12,000 g for 30 min. The protein concentration was determined by a bicinchoninic acid assay kit (Pierce Chemical, Rockford, IL). A total of 60 μg of protein lysates from each sample were added into the loading buffer, mixed, and heated at 95°C for 5 min. Then the samples were loaded into sodium dodecyl sulfate polyacrylamide electrophoresis gels (8%) and transferred to polyvinylidene fluoride membranes (Bio-Rad, Hercules, CA). The resultant blots were blocked in Tris-buffered saline/Tween 20 buffer (10 mmol/L Tris, 150 mmol/L NaCl, 0.1% Tween-20, pH 8.0) with 5% milk for 1 h. The membranes were then incubated with the indicated primary antibodies (rabbit anti-mGluR5, 1:2,000, or rabbit anti-PSD-95, 1:2,000, or mouse anti-SAP102, 1:1,000, or mouse anti-β-actin, 1:5,000) at 4°C overnight. Next, the membranes were rinsed with Tris-buffered saline/Tween 20 for 3 times and incubated with appropriate secondary antibodies for 1 h at room temperature. Finally, the membrane was briefly washed and subjected to enhanced chemiluminescence exposure (GE Healthcare, Buckinghamshire, UK). Images were obtained on a computer-aided CCD camera. Optical density was detected with Mercator software (Explora Nova).

Ultrastructural Examination

To examine the ultrastructural synaptic changes in the striatum, rats were sacrificed following the last delivery of drug and perfused with 0.9% NaCl saline solution followed by 3% paraformaldehyde containing 0.4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). In brief, the tissues from the dorsal part of the striatum were submerged in a solution (2% osmium tetroxide) for 30 min, rinsed 3 times in distilled water, dehydrated in graded ethanol solutions, and finally embedded in araldite. Ultrathin slices from the outer surface of each striatal vibration sections were gathered onto grids. Following counterstaining with lead citrate, the ultrastructural alterations of the thin slices were detected and photographed by using transmission electron microscope (JEOL, Tokyo, Japan).

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). All analyses were carried out with the SPSS 18.0 version (SPSS Inc., Chicago, IL). To evaluate the interaction of treatment and time on the abnormal movements, we performed repeated measures analysis of variance (ANOVA) followed by post hoc analysis with the Bonferroni's correction. For comparisons in protein expression and synaptic changes among groups, we performed ANOVA followed by the Tukey's post hoc analysis. For comparisons in proportion of perforated synapses, we carried out chi-squared test. The value of $p < .05$ was considered as statistically significant.

Results

MPEP Attenuates AIM Induced by L-DOPA

To mimic dyskinesia of PD patients, we consecutively treated PD model rats with L-DOPA for 21 days and observed progressive AIMs. Next, we examined the potential role of mGluR5 in AIMs. A repeated-measures ANOVA revealed a significant time and treatment interaction effect in axial AIM score, $F(2, 18) = 4.82$, $p = .012$ (Figure 1(a)), limb AIM score, $F(2, 18) = 5.51$, $p = .002$, total AIM score, $F(2, 18) = 4.42$, $p = .04$ (Figure 1(d)), but no significant time and treatment interaction effect in orolingual AIM score, $F(2, 18) = 3.4$, $p = .14$ (Figure 1(c)). Specifically, Day 21 after L-DOPA application, the axial AIM score of MPEP plus L-DOPA group was markedly reduced than that of L-DOPA treatment group ($p = .002$; Figure 1(a)). Similarly, the limb AIM score of the coadministration group was remarkably decreased at Days 11, 18, and 21 as compared with the L-DOPA treatment group ($p < .001$, $p < .001$, $p = .004$, respectively; Figure 1(b)). The total AIM score of the coadministration group was significantly decreased on the 21st day, as compared with the L-DOPA treatment group ($p = .014$; Figure 1(d)). Lesioned animals with injection of saline (controls, n = 10) or MPEP (n = 10) alone failed to develop any AIM. Together, these results suggested that inhibition of mGluR5 by MPEP prevents L-DOPA-induced AIM in PD model rats.

MPEP Prevents the Decrease of L-DOPA-Induced Rotational Response Duration

Previous studies showed that the reduction in the rotational response duration induced by L-DOPA reflects a motor impairment in PD model rats (Lundblad et al., 2003). A significant interaction of time and treatment was observed in rotational response duration, $F(2, 18) = 6.61$, $p < .001$ (Figure 1(e)). Consistent with previous studies, we found that administration of L-DOPA alone, delivered twice daily for 21 days, substantially decreased the rat rotational response duration ($p < .001$, Figure 1(e)). By contrast, the L-DOPA-induced duration of rotational response remained unchanged across the 21-day observation period in the L-DOPA plus MPEP group ($p > .05$; Figure 1(e)), suggesting the blocking role of mGluR5 inhibition by MPEP. The difference between the coadministration group and the L-DOPA alone group was significant on
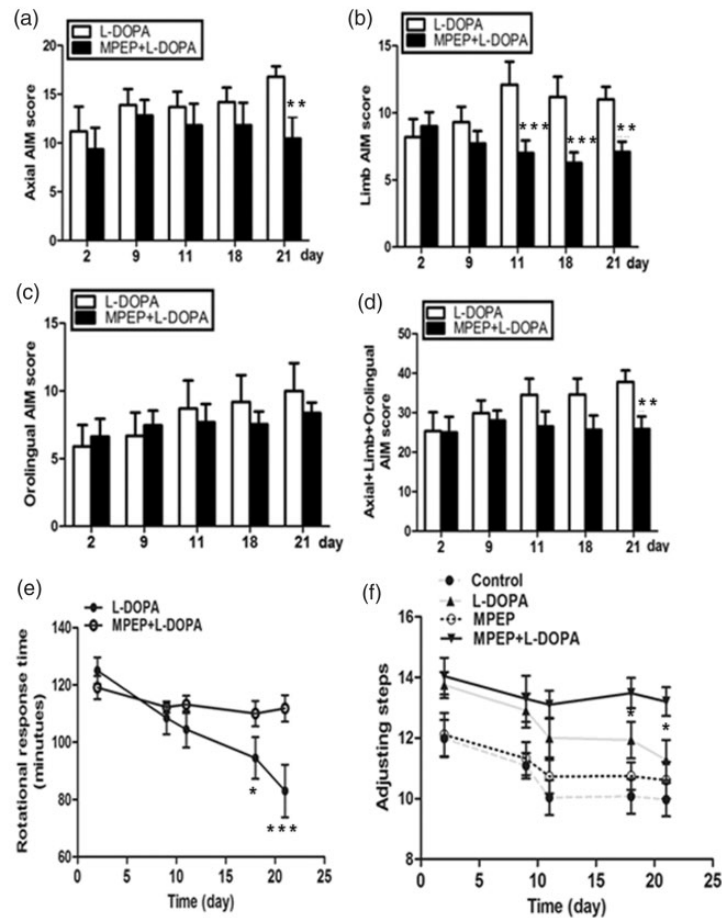
Figure 1. Effects of MPEP on L-DOPA-induced abnormal movements in 6-OHDA-lesioned rats. (a) Axial AIM scores are shown at time points as indicated. (b) Limb AIM scores are shown. (c) Orolingual AIM scores are shown. (d) Total (axial, limb, and orolingual) AIM scores are shown. (e) Rotational response durations are shown. (f) Forepaw adjusting steps are shown. AIM scores, rotational response durations, and forepaw adjusting steps were recorded at 2, 9, 11, 18, and 21 days after L-DOPA application. Rats were daily treated with L-DOPA alone or with MPEP 30 min before L-DOPA for 3 weeks. Data were obtained from 10 animals for each group and are shown as mean$\pm$ SEM. *$p < .05$, **$p < .01$, ***$p < .001$ versus L-DOPA-treated group. AIM = abnormal involuntary movement; MPEP = 2-methyl-6-(phenylethynyl)pyridine; L-DOPA = levodopa.

the 15th and 21st day ($p = .024$, $p < .001$, respectively, Figure 1(e)). Lesioned animals receiving an injection of saline (controls, n $= 10$) or MPEP (n $= 10$) alone failed to exhibit rotational responses. Thus, these findings implicate that inhibition of mGluR5 by MPEP blocks L-DOPA-induced reduction in the rotational response duration in PD model rats.

MPEP Blocks the Decrease of Forepaw Adjusting Steps Induced by L-DOPA

Next, we further assessed forelimb akinesia by the left forepaw adjusting steps (Olsson et al., 1995). A repeated-measures ANOVA revealed a significant time and treatment interaction effect in forepaw adjusting steps, $F(2, 36) = 4.56$, $p = .016$ (Figure 1(f)). The L-DOPA-treated PD model animals showed an obvious increase in left forepaw adjusting steps at the beginning, as compared with saline-treated rats. However, on the 18 and 21 days after L-DOPA application, the forepaw adjusting steps gradually decreased ($p = .042$; $p = .031$, respectively, Figure 1(f)). Such a decrease was greatly blocked by coadministration of L-DOPA and MPEP (Figure 1(f)), suggesting the antagonistic effect of MPEP. Moreover, administration of MPEP alone appeared not to affect the forepaw adjusting steps.

## MPEP Attenuates the Upregulation of Striatal mGluR5 Expression Induced by L-DOPA

The aforementioned behavioral results suggested the overactivity of mGluR5 signal in PD model rats.

In addition, there is evidence showing the overexpression of mGluR5 in the basal ganglia of PD model monkeys with LID and in PD patients with motor complications (Samadi et al., 2008; Ouattara et al., 2010, 2011; Morin et al., 2013a, 2013c). Thus, we next examined the expression of mGluR5 at the striatum. Western blot analysis revealed that delivery with L-DOPA markedly upregulated the striatal mGluR5 expression in the lesioned side ($p = .014$, as compared with the saline group, Figure 2 (a)). The upregulation was largely inhibited by coapplication with MPEP ($p = .025$, as compared with the L-DOPA group, Figure 2(a)). In addition, application of MPEP alone showed a reduction in expression of mGluR5 (Figure 2(a)). Collectively, these data suggested

overexpression of mGluR5 in PD model rats and the inhibitory role of MPEP.

MPEP Blocks Upregulation of L-DOPA-Induced Striatal Postsynaptic Protein Expression

It is well known that PSD-95 and SAP102 are two major postsynaptic scaffolding proteins in the excitatory PSD (Yao et al., 2004; Porras et al., 2012; Murata and Constantine-Paton, 2013), especially at postsynaptic dendritic spines in the striatum (Chen et al., 2015). There is evidence that the level of PSD-95 expression is remarkably upregulated in the unilateral 6-OHDA-lesioned rat model administered by L-DOPA (Nash
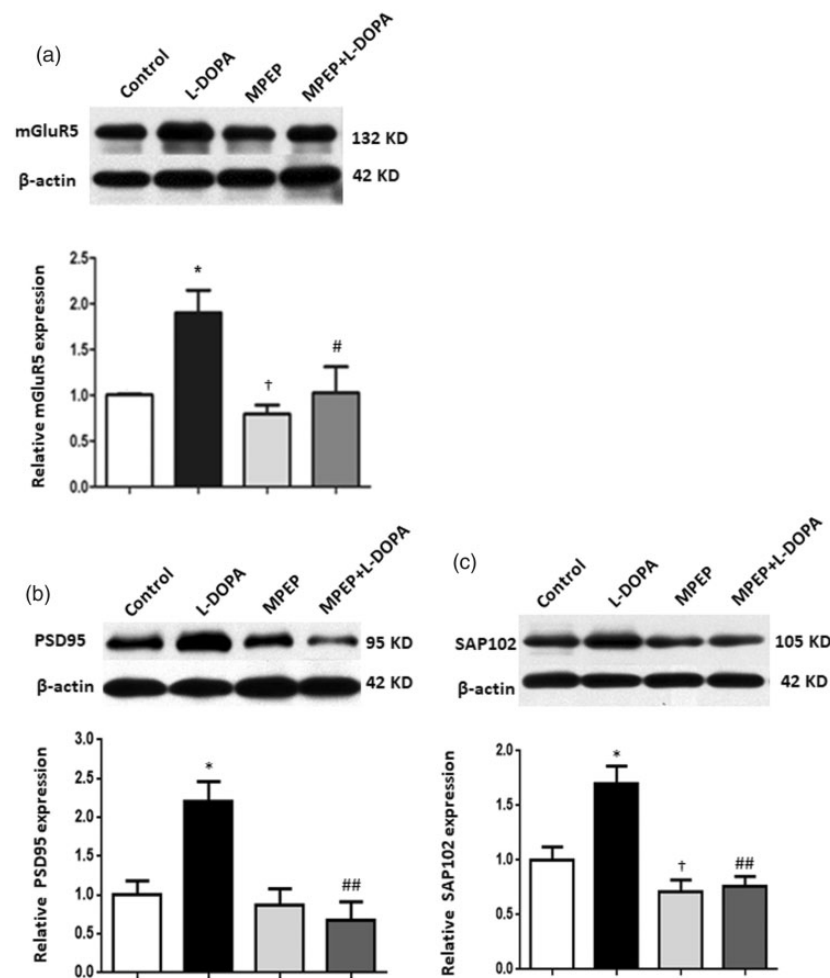

Figure 2. MPEP reversed the L-DOPA-induced increase in striatal mGluR5 expression and postsynaptic protein (PSD-95 and SAP 102) expression in 6-OHDA-lesioned side. The proteins were analyzed from 6-OHDA-lesioned rats with saline treatment, or L-DOPA treatment, or MPEP treatment, or L-DOPA in combination with MPEP treatment. (a) The expression of mGluR5 in four groups. (b) The expression of PSD-95 in four groups. (c) The expression of SAP102 in four groups. Data are obtained from four animals for each group and are represented as mean ±SEM. Upper panel, representative blots; Lower panel, quantitative analysis. *p < .05 versus control group (6-OHDA-lesioned alone) or versus L-DOPA-treated group.*p < .05 versus the saline group; †p < .05 versus the saline group; #p < .05, ##p < .01 versus the L-DOPA-treated group. MPEP = 2-methyl-6-(phenylethynyl)pyridine; mGluR5 = metabotropic glutamate receptor type 5; PSD = postsynaptic density; L-DOPA = levodopa.

et al., 2005). Thus, we further examined PSD-95 expression at the lesioned striatum. Western blot analysis showed that administration with L-DOPA increased PSD-95 expression in the lesioned striatum ($p = .028$, as compared with the saline group, Figure 2(b)). We next observed that the increase was largely inhibited by coadministration of L-DOPA and MPEP ($p = .004$, as compared with the L-DOPA group, Figure 2(b)). Similar results were observed in the expression of SAP102 (Figure 2(c)). Administration of MPEP alone seemed not to affect PSD-95 and SAP102 expression. Therefore, these data suggested the blocking effect of mGluR5 inhibition by MPEP on L-DOPA-induced striatal postsynaptic protein overexpression in PD rats.

MPEP Reverses L-DOPA-Induced Ultrastructural Changes

Previous reports showed that LID is associated with aberrant synaptic plasticity in the striatum (Cenci and Lundblad, 2006; Jenner, 2008; Blandini and Armentero, 2012). Thus, we then examined the change in synaptic structures in the lesioned dorsal striatum by using trans-
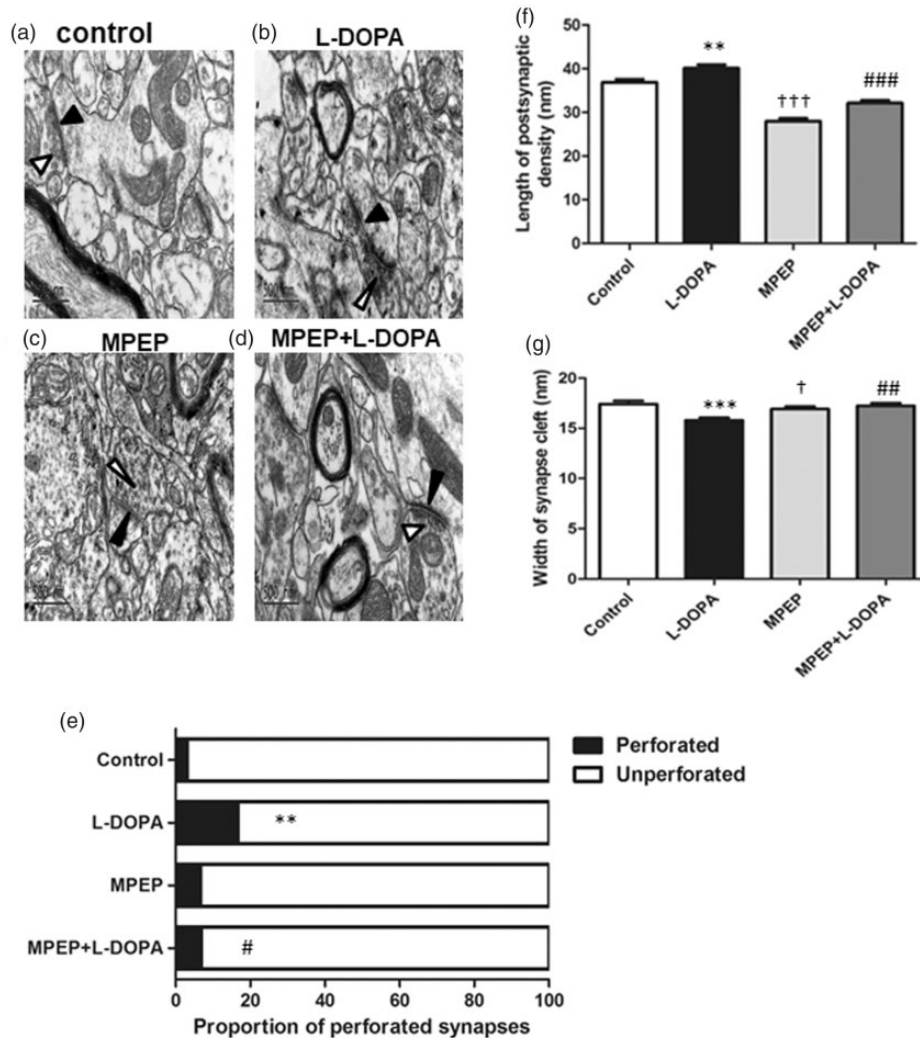
Figure 3. Effect of MPEP on the L-DOPA-induced ultrastructural changes of synapses in the 6-OHDA-lesioned striatum of rat. The black arrows indicate postsynaptic density and the white arrows indicate synaptic vesicles in the presynaptic membrane. The micropictures showed the representative changes in striatal synapses in the rats treated with saline (a), L-DOPA (b), MPEP (c), or MPEP plus L-DOPA (d). Scale bars = 500 nm. (e), (f), and (g) show quantitative changes of the proportion of perforated synapses, postsynaptic density length, and synapse cleft width, respectively. Data were obtained from five animals for each group and are represented as mean ±SEM. **$p < .01$, ***$p < .01$ versus the saline group; †$p < .05$, †††$p < .001$ versus the saline group; #$p < .05$, ##$p < .01$, ###$p < .001$ versus the L-DOPA-treated group. MPEP = 2-methyl-6-(phenylethynyl)pyridine; L-DOPA = levodopa.

mission electron microscope. As seen in Figure 3(a) to (d), there were both continuous and perforated synapses in terms of the continuity of PSD. We found that the proportion of perforated synapses was higher in the L-DOPA group relative to the control group ($p = .005$; Figure 3(e)). Coadministration of L-DOPA and MPEP reduced the proportion ($p = .032$, Figure 3(e)). To further examine the changes in synapses, we quantitatively measured the length of PSD and the width of synaptic cleft. Our results showed a thicker PSD length and a narrower synaptic cleft width in the L-DOPA-administered rats relative to the control group ($p = .006$, $p < .001$, respectively, Figure 3(f) and (g)). However, systemic coadministration of L-DOPA and MPEP attenuated these postsynaptic effects ($p < .001$, $p = .002$, as compared with the L-DOPA group, Figure 3(f) and (g)). Moreover, the administration of MPEP alone significantly decreased the length of PSD and the width of synapse cleft relative to the saline group ($p < .001$, $p = .041$; Figure 3(f) and (g)). These results suggested that MPEP reverses L-DOPA-induced ultrastructural changes in the PD animals.

Discussion

Glutamate is the most abundant excitatory neurotransmitter in the brain, serving most of the synaptic transmission in the central nervous system (Meldrum, 2000; Platt, 2007). It was reported that glutamatergic transmission is elevated in the basal ganglia in PD (Klockgether and Turski, 1993) and LID (Chase and Oh, 2000; Calon et al., 2003). There is evidence that the therapeutic use of an mGluR5 antagonist led to normalization of glutamate neurotransmission in the PD brain and prevented the occurrence of LID (Morin et al., 2013c). Previous studies showed that LID can be viewed as a result of aberrant dopamine-related neural plasticity at glutamate corticostriatal synapses in the striatum (Graybiel, 2004). Consistent with the notion, our results showed that motor complications induced by administration of L-DOPA are accompanied by the enrichment of the corticostriatal synaptic ultrastructures and signaling proteins in 6-OHDA-lesioned hemiparkinsonian rats, while coadministration of L-DOPA and MPEP ameliorated motor complications with a reduction in the enrichment of ultrastructures and the expression of postsynaptic proteins (PSD-95 and SAP102).

Forepaw adjusting steps and rotational response duration are commonly used to assess the antiparkinsonian effect of candidate compounds. Previous studies showed that the forepaw adjusting steps was reduced in PD rats after L-DOPA replacement therapy for a certain period (Chang et al., 1999). In this study, we found that coapplication of L-DOPA with MPEP eliminated the decreasing tendency of left forepaw adjusting steps, suggesting

that MPEP strengthened L-DOPA antiparkinsonian effects. These results are in agreement with a previous study performed in MPTP-lesioned monkeys (Oh and Chase, 2002; Morin et al., 2010, 2013a).

Our results showed that the PD model rats delivered with L-DOPA showed a progressive reduction in the rotational response duration, similar to the "wearing-off" phenomena in PD patients (Henry et al., 1998; Oh and Chase, 2002). Importantly, coadministration of L-DOPA with MPEP prolonged the duration of rotational response across 21st day. Moreover, a satisfactory tolerability of MPEP was observed due to persistent amelioration even following MPEP administration. These results are in accordance with a previous study (Johnston et al., 2010), suggesting that combination of L-DOPA with MTEP caused an elevation (of 37%) in "good" on-time relative to L-DOPA alone, such duration of "good" on-time was remarkably greater than in vehicle-injected animals.

The 6-OHDA or MPTP-lesioned animals treated with L-DOPA exhibited AIM, equivalent to the "peak-dose" dyskinesia in L-DOPA-administered PD patients (Cenci and Lundblad, 2006). Our data showed that the rats delivered with L-DOPA alone displayed onset and progressive increase in AIM. But coadministration of L-DOPA with MPEP decreased limb scores and axial and total AIM. Consistently, there are reports showing that selective mGluR5 antagonist MPEP blocked the occurrence of LID and kept antiparkinsonian effects in MPTP monkeys or 6-OHDA-lesioned rats (Morin et al., 2010, 2013a). Furthermore, acute or chronic application of selective mGluR5 antagonist (Fenobam) also reduced the occurrence of peak-dose dyskinesia without influencing L-DOPA antiparkinsonian effects and prolonged the L-DOPA motor stimulant effects in 6-OHDA-lesioned rats and MPTP-treated monkeys. (Rylander et al., 2010).

The underlying mechanisms for the effect of mGluR5 antagonists on LID are still largely uncertain. Our results showed that administration of L-DOPA enhanced mGluR5 expression in the striatum. Interestingly, coadministration of L-DOPA with MPEP to PD rats not only attenuated AIM but also dampened overexpression of mGluR5 in the striatum comparable to the level of the 6-OHDA-lesioned group, consistent with a previous report (Morin et al., 2013b) that mGlu5 receptor-specific bindings in the basal ganglia were boosted in L-DOPA-administered MPTP monkeys as compared with controls but not in the animals cotreated with L-DOPA and MPEP. Furthermore, an increase in mGlu5 receptor expression was found in the striatum of dyskinetic MPTP-lesioned monkeys and in parkinsonian patients with motor complications (Ouattara et al., 2010, 2011). Together, combined with previous studies, our results suggested that higher mGluR5 expression is

correlated with the occurrence of L-DOPA-induced motor complications.

Previous studies showed that LID may be due to aberrant brain plasticity (Graybiel, 2004). By using electron microscopy techniques, we examined the morphologic changes in corticostriatal synaptic ultrastructures and signaling proteins in the striatum of PD or LID rats. Our data showed much thicker postsynaptic densities, narrower synapse cleft, as well as the increased ratio of perforated synapses in the L-DOPA-treated lesioned side, while coadministration of L-DOPA and MPEP reversed these postsynaptic effects. These findings suggested that the L-DOPA treatment leads to more active synapses, especially in the postsynaptic components, in line with one prior study (Picconi et al., 2003) reporting an abnormal type of corticostriatal synaptic plasticity in acute brain slices from L-DOPA-treated models. More importantly, the selective blockade of mGluR5 in combination with L-DOPA treatment in striatal neurons prevented the postsynaptic effects.mGluR5 is abundant in the basal ganglia structures, particularly at postsynaptic membranes of striatal neurons (Litim et al., 2016; Sebastianutto and Cenci, 2018), its inhibition is thought to modulate postsynaptically excitatory synaptic transmission and glutamate hyperactivity in PD and LID (Conn et al., 2005). However, how these postsynaptic morphological alterations influence the contribution of mGluR5 to LID symptoms needs to be further investigated.

It is believed that PSD-95 and SAP102, as key postsynaptic components, are involved in synaptic activity strength (Chen et al., 2011, M. Liu et al., 2018). To examine the roles of the two proteins in aberrant synaptic plasticity, we measured the expression of PSD-95 and SAP102 in the striatum in LID rats. Our findings suggested a marked increase in the expression of PSD-95 and SAP102 by the administration of L-DOPA in the striatum. However, coadministration of L-DOPA with MPEP to PD rats not only alleviated L-DOPA-induced motor complications but also dampened overexpression of striatal PSD-95 (consistent with a past study, Porras et al., 2012) and SAP102 protein to the level of the 6-OHDA-lesioned group. Porras et al. showed that PSD-95 expression in the putamen was remarkably upregulated in the putamen of the MPTP-lesioned monkeys relative to controls. PSD-95 expression and its subcellular distribution were both changed in the striatum of 6-OHDA-lesioned rats administered by L-DOPA, with an increased expression and enrichment at the synaptic site (Nash et al., 2005). Although there is no report about the role of SAP102 in PD, as the postsynaptic component, its expression exhibited a similar profile to PSD-95 expression under these experimental conditions. Taken together, we speculated that upregulation of PSD-95 and SAP102 expression in PSD induced by L-DOPA may

exert important effect on the occurrence of LID. Nevertheless, how mGluR5 affect the hyperactivity of the corticostriatal synaptic signaling proteins in LID needs to be further determined.

Notably, although application of MPEP alone did not cause significantly abnormal movement behavior, the proportion of perforated synapses, PSD-95 expression, and MPEP itself blocked the expression of mGluR5 and SAP102 as well as the length of PSD and the width of synaptic cleft.

In summary, this study suggested that the selective mGluR5 antagonist MPEP reversed the decreasing tendency of left forepaw adjusting steps, prolonged the rotational response duration, alleviated dyskinesia, and reduced overexpression of mGluR5 in the lesioned striatum of LID rats. Further, MPEP inhibited the hyperactivity of striatal synaptic ultrastructure and postsynaptic proteins (PSD-95 and SAP102) induced by L-DOPA treatment. Therefore, these results provide evidence that aberrant glutamate-related neural plasticity at corticostriatal synapses in the striatum is tightly correlated with the occurrence of LID, and specific inhibition of mGluR5 by MPEP alleviates LID in the PD rats.

Declaration of Conflicting Interests

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