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Effects of the prototypical $mGlu_5$ receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats

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Abstract

In the present study, we evaluated the effect of the prototypical metabotropic glutamate receptor 5 ($mGlu_5$) antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) on motor behaviour in rats using the accelerating rotarod, spontaneous locomotor activity and the 6-hydroxy-dopamine (6-OHDA) lesion model to assess its treatment potential for Parkinson's disease. The data indicate that MPEP at doses between 7.5 and 300 mg/kg, p.o. did not disrupt endurance performance on the accelerating rotarod (4–40 rpm in 300 s) which indicates that MPEP has a relatively high safety margin. However, while ineffective at doses of 3.75, 7.5 and 15 mg/kg (p.o.) MPEP inhibited spontaneous locomotor activity at doses of 30 and 100 mg/kg (p.o.). In the 6-OHDA rat rotation model, at doses of 7.5, 15 and 30 mg/kg (p.o.), MPEP induced a dose-dependent ipsilateral rotational response that reached statistical significance at the highest dose tested. This effect was relatively small but consistent. In combination with direct or indirect dopamine agonists, i.e. apomorphine (0.25 mg/kg, s.c.) and D-amphetamine (2.5 mg/kg, i.p.), MPEP (7.5, 15 or 30 mg/kg, p.o.) was found to significantly inhibit these dopamine receptor mediated rotational responses. MPEP injected at a dose of 30 mg/kg also inhibited the rotational response induced by L-DOPA (25 mg/kg, i.p.). (+)MK-801 was used in these rotation experiments as the reference compound. In view of these findings, it could be concluded that MPEP and potentially other $mGlu_5$ receptor antagonists are probably not appropriate drug candidates for the symptomatic treatment of Parkinson's disease.

Keywords: Glutamate; Receptor; Metabotropic; Antagonist; 2-Methyl-6-(phenylethynyl)-pyridine (MPEP)

1. Introduction

Metabotropic glutamate receptors are a family of G-protein-coupled receptors linked to multiple second messengers and modulation of ion channel functions in the nervous system (Knöpfel et al., 1995; Conn and Pinn, 1997). Molecular cloning has revealed the existence of eight distinct receptor subtypes, termed $mGlu_1$–$mGlu_8$, which are classified into three subgroups based on sequence similarities, pharmacological profiles and signal transduction pathways activated in heterologous systems. Group I receptors ($mGlu_1$ and $_5$) couple to phospholipase C and regulate neuronal excitability whereas group II ($mGlu_2$ and $_3$) and group III receptors ($mGlu_4$, $_6$, $_7$, $_8$) inhibit adenylyl cyclase and modulate neurotransmitter release.

Metabotropic glutamate receptors have been proposed as potentially new therapeutic targets for a number of neurological and psychiatric disorders (Knöpfel et al., 1995; Conn and Pinn, 1997; Nicoletti et al., 1997). However, these speculations are largely based on the expression pattern of distinct mGlu-subtype receptors in the central nervous system and on the effects of non-selective compounds that fail to discriminate between distinct receptor subtypes. The recent discovery of a series of potent, selective and systemically active antagonist for the $mGlu_5$ receptor opened now the possibility to investigate the role of this receptor subtype in behaviour and disease states (Gasparini et al., 1999; Varney et al., 1999). The most potent derivative of this series, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), completely inhibited quisqualate-stimulated phosphoinositide hydrolysis with an IC_{50} value

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of 36 nM whilst devoid of agonist or antagonist activities at any of the other mGlu receptor subtypes up to 100 μM (Gasparini et al., 1999). Upon testing in rodents it was found that MPEP is readily oral bioavailable and easily penetrates the brain reaching relatively high brain levels shortly (within 60 min) following oral application (Spooren et al., unpublished observation).

The $mGlu_5$ receptor is widely distributed in the central nervous system with particularly high expression in hippocampus and the striatal areas such as the nucleus accumbens and caudate putamen. However, $mGlu_5$ receptors are also expressed in the output structures of the striatum, i.e. the internal and external pallidal segments and the substantia nigra pars reticulata (Testa et al., 1994). These brain areas are well-known as key elements in the basal ganglia circuitry and, therefore, to play an important role in behaviour and disease such as in Parkinson's disease (Albin et al., 1989).

A widely used model to evaluate the potential antiparkinson activity of experimental therapeutics is the so-called hemiparkinsonian rat or the unilateral 6-OHDA lesioned rat (Ungerstedt, 1971; for review: Schwarting and Huston, 1996a,b). The model involves the unilateral injection of 6-OHDA into the medial forebrain bundle which induces extensive loss of dopaminergic cells in the substantia nigra pars compacta (Schwarting and Huston, 1996a,b). The resulting imbalance in dopamine innervation between the striata produces postural asymmetry. Dopamine releasing agents such as amphetamine exacerbate the dopamine imbalance that favors the non-lesioned side and thus produces ipsilateral rotations (Schwarting and Huston, 1996a,b). In contrast, direct agonists such as apomorphine evoke contralateral rotations reflecting an action at supersensitive denervated dopamine receptors ipsilateral to the lesion (Schwarting and Huston, 1996a,b). In the present study, we have used the 6-OHDA model to investigate the therapeutic potential of the novel prototypical $mGlu_5$ receptor antagonist MPEP for the symptomatic treatment of Parkinson disease. We here describe the effect of MPEP alone as well as when given in a combination with apomorphine, D-amphetamine and L-DOPA. In addition, the effect of MPEP on motor coordination and motor activity was further investigated using the accelerating rotarod and spontaneous locomotor activity, respectively.

2. Materials and methods

2.1. Rotarod and locomotor activity experiments

2.1.1. Animals

Male Wistar rats (Iffa Credo, Les Oncins, France; $n = 222$), 100–120 g were used. The animals were housed four per cage in a temperature controlled room (22 ± 1°C) under artificial illumination (6:00–18:00 h, lights on) with access to water and food (Ecosan, Eberle Nafag, Gossau, Switzerland), ad libitum.

2.1.2. Rotarod

Drug-naïve animals were trained twice daily on two successive days on the accelerating rotarod (4–40 rpm in 300 s; TSE, Bad Homburg, Germany). On the test day (day 3), the animals received an injection of MPEP at low doses (7.5, 15, or 30 mg/kg, p.o.; $n = 12$ per treatment group) or vehicle (methylcellulose (0.5%); Animed, Allschwil, Switzerland) or an injection of MPEP at high doses (100, 200 or 300 mg/kg, p.o.), baclofen (10 mg/kg, p.o., i.e. a dose known to reduce endurance performance; Spooren et al. unpublished observation) as the positive control, or vehicle. The animals were then repeatedly tested for their endurance performance on the accelerating rotarod (longest time spent on the rotarod; cut of time 300 s) 1, 3, 6 and 24 h after application.

2.1.3. Locomotor activity

Drug-naïve non-habituated rats received an injection with MPEP at doses 3.75, 7.5, 15, 30 or 100 mg/kg, p.o., or the vehicle (methylcellulose (0.5%); $n = 18$ per treatment group) and they were subsequently placed in locomotor activity cages ($17 \times 32 \times 20$ cm; Motron motility, Novartis Pharma, Basel, Switzerland) and the number of beam interruptions (vertical and horizontal) was registered in 10-min intervals for 120 min.

2.2. 6-OHDA rat rotation experiments

2.2.1. Animals

Male Sprague–Dawley Rats (Iffa Credo, Les Oncins, France; $n = 120$), 250–280 g at the time of surgery (see below) were used. The animals were housed four per cage in a temperature controlled room (22 ± 1°C) under artificial illumination (6:00–18:00 h, lights on) with access to water and food (Ecosan, Eberle Nafag), ad libitum.

2.2.2. Surgery

Procedures were outlined previously (Spooren et al., 1999) and adapted from Nitsch et al. (1993). Briefly, before surgery all animals received an injection of desipramine hydrochloride (30 mg/kg, i.p.; USPC, Rockville, USA) to protect noradrenergic cells. One hour later the animals received an injection of pentobarbital (55 mg/kg, i.p., Vetanarcol, Veterinaria, Zurich, Switzerland) and the animals were subsequently placed (under deep anesthesia) in a UHL stereotaxic apparatus. A unilateral lesion was made by injecting 9 μg 6-OHDA (6-OHDA-hydrobromide; Fluka Chemie, Buchs, Switzerland) in 0.7 μl ascorbic acid solution (dilution: 1 mg/ml) over 10 min into the left medial forebrain bundle (coordinates: AP 3.6 mm (from bregma), L 1.1 mm (from midline) and H 7.9 mm (from dura; Pellegrino et al., 1979). The injection was

aimed at the rostral pole of the substantia nigra where the ascending nigrostriatal bundle converges. Accordingly, a maximum number of dopaminergic neurons can be lesioned by injecting into this particular site, resulting in a so-called near maximal lesion (Hudson et al., 1993). Following the injection, the needle was kept in place for another 10 min to allow diffusion of the toxin away from the injection site and to prevent back-flow.

2.2.3. Animal selection

Following surgery the animals were allowed to recover for at least 21 days before testing them in the rotameter. Selection of animals to be included in the studies was performed using the rotational response to apomorphine (0.25 mg/kg, s.c.) and only responders (> 100 net rotations) to this treatment were further used. The selected animals were used in subsequent experiments and the washout period between such experiments was at least 7 days. The animals were randomized for each new drug challenge.

2.2.4. Rotameter equipment

All animals were tested in automated rotameter cylinders (TSE, Bad Homburg, Germany) and the number of rotations (ipsilateral and contralateral) were automatically recorded.

2.2.5. Rotameter testing

2.2.5.1. MPEP alone. The animals were allowed to habituate to the rotameter bowls for 15 min. Subsequently, the animals received an injection of MPEP (doses: 7.5, 15 or 30 mg/kg, p.o.), the vehicle (methylcellulose (0.5%)) or (+)MK-801 (0.3 mg/kg, i.p.; a dose with a clear rotation response; Mele et al., 1998), i.e. the reference compound in these experiments (Clineschmidt et al., 1982). Following injection, the number of rotations was automatically recorded for 120 min.

2.2.5.2. Combined injections. Following an injection with either MPEP (doses: 7.5, 15 or 30 mg/kg, p.o.), the vehicle (methylcellulose (0.5%)) or (+)MK-801 (0.3 mg/kg, i.p.), the animals were placed in the rotameter bowls and allowed to habituate for 30 min. Subsequently, the animals received an injection of either apomorphine (0.25 mg/kg, s.c.), d-amphetamine (2.5 mg/kg, i.p.) or L-DOPA (25 mg/kg, i.p.). Following this injection, the number of rotations was automatically recorded for the next 90 to 240 min, depending upon the specific requirements of the experiment. An additional group was included in each experiment that was treated identically to the procedures outlined above but was injected with vehicle in order to determine the number of spontaneous rotations, i.e. the absolute control (abs).

2.2.6. Statistics

2.2.6.1. Rotarod experiment. Endurance performance on the rotarod was analysed using a two-factor repeated measures ANOVA (analysis of variance) with factors dose and time (repeated factor). Distinct time-points were compared using the Student's *t*-test (baclofen only).

2.2.6.2. Locomotor activity experiment. Activity counts were statistically evaluated using an ANOVA with factors dose and activity counts (horizontal and vertical) followed by Dunnett's test for multiple comparison of different dose levels with a control test (vehicle).

2.2.6.3. Rotation experiments. Statistical analysis was performed on the number of net rotations (ipsilateral-contralateral or vice versa) using an ANOVA followed by Dunnett's test, for multiple comparison of different dose levels (MPEP), or Student's *t*-test where appropriate ((+)MK-801; software: SYSTAT 8.0®).

3. Results

3.1. Rotarod experiments

MPEP induced no significant change in endurance performance when compared to controls on the accelerating rotarod at low dose range between 7.5 and 30 mg/kg, p.o. (ANOVA, $F = 0.096$, $p > 0.05$; Table 1a) nor at high-dose range between 100 and 300 mg/kg, p.o. (ANOVA, $F = 0.190$, $p > 0.05$; Table 1b) 1, 3, 6 or 24 h (high dose range only) after application. In contrast, baclofen, i.e. the positive standard, at a dose of 10 mg/kg, p.o. reduced the endurance performance to ±50% of controls ($P < 0.001$) 1 h after application. Endurance performance was again normalised 3 h after application.

3.2. Locomotor activity experiments

MPEP significantly reduced spontaneous horizontal ($F = 4.217$, $P < 0.01$) and vertical locomotor activity ($F = 5.512$, $P < 0.001$; Table 2). Although only the low dose of 7.5 mg/kg MPEP non-significantly increased locomotor activity, the high dose of 100 mg/kg MPEP significantly reduced horizontal locomotor activity (−38%; $P < 0.05$). Vertical activity was significantly reduced at doses of 30 and 100 mg/kg (−57%; $P < 0.05$ and −73%; $P < 0.001$, respectively).

3.3. 6-OHDA rotation experiments

3.3.1. MPEP-alone

MPEP increased the number of (ipsilateral) net rotations in a dose-dependent manner (ANOVA, $F = 5.940$, $P <$

Table 1
Accelerating rotarod

(a) Effect of MPEP (low doses)

Drug	Dose (mg/kg)	Endurance (s)				
		1 h	3 h	6 h	24 h	after application
Vehicle	0	254	267	280	nd	s
		14	13	11		S.E.M.
MPEP	7.5	270	258	257	nd	s
		15	12	15		S.E.M.
	15	256	262	279	nd	s
		19	17	11		S.E.M.
	30	244	280	287	nd	s
		16	11	6		S.E.M.

Mean (±S.E.M.) endurance performance on the accelerating rotating rotarod (4–40 rpm in 300 s) in rats ($n = 15$ per treatment group) at distinct time points (1, 3 and 6 h) after application of MPEP at doses of 7.5, 15 or 30 mg/kg, p.o. or vehicle (methylcellulose (0.5%)). nd = not determined

(b) Effect of MPEP (high doses)

Vehicle	0	244	274	281	283	s
		19	12	9	11	S.E.M.
MPEP	100	242	255	282	274	s
		19	20	12	13	S.E.M.
	200	249	256	268	242	s
		18	22	16	17	S.E.M.
	300	238	259	258	280	s
		25	22	13	14	S.E.M.
Baclofen	10	133[a]	268	296	288	s
		16	17	2	6	S.E.M.

Mean (±S.E.M.) endurance performance on the accelerating rotating rotarod (4–40 rpm in 300 s) in rats ($n = 12$ per treatment group) at distinct time points (1, 3, 6 and 24 h) after application of MPEP at doses of 100, 200, 300 mg/kg, p.o., baclofen 10 mg/kg, p.o. or vehicle (methylcellulose (0.5%))

[a] $P < 0.001$ vs. vehicle (0 mg/kg).

0.002). Doses of 7.5 and 15 mg/kg (p.o.) may be considered as ineffective; in contrast, the dose of 30 mg/kg significantly increased the number of net rotations ($P < 0.001$, Fig. 1). Although statistically significant, the rotational behaviour induced by MPEP was completely different from that seen with (+)MK-801 (0.3 mg/kg, i.p.), i.e. the positive standard, and it was characterized by a slow and intermittent rotational response which tended to continue beyond the selected cut-off time of 120 min. Furthermore, quantitatively the increase in net rotations was small when compared to the effect of (+)MK-801 (0.3 mg/kg, i.p.). The latter increased net rotations six times more than that induced by 30 mg/kg MPEP (Fig. 1).

Table 2
Locomotor activity

Drug	Dose (mg/kg)	# Activity counts (mean ± S.E.M.)		
		horizontal	vertical	
MPEP	0	3555 ± 409	628 ± 95	counts
	3.75	3432 ± 287	505 ± 60	counts
	7.5	4482 ± 508	699 ± 115	counts
	15	3337 ± 439	517 ± 161	counts
	30	2816 ± 283	270 ± 44[a]	counts
	100	2197 ± 233[a]	169 ± 47[b]	counts

Mean (±S.E.M.) number of activity counts in the horizontal and vertical dimension in 120 min of registration following an injection with MPEP in the doses of 3.75, 7.5, 15, 30 or 100 mg/kg, p.o. or vehicle (methylcellulose (0.5%)). $n = 18$ per treatment group.

[a] $P < 0.05$.

[b] $P < 0.001$ vs. vehicle.

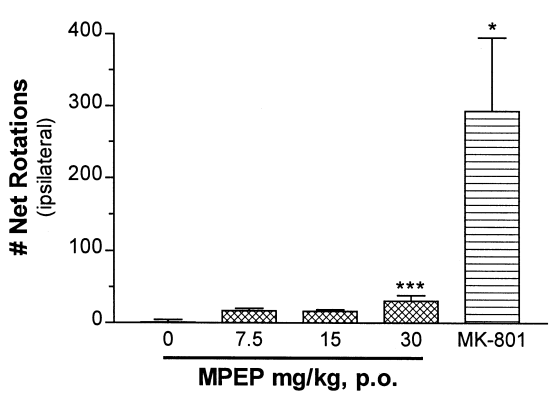

Fig. 1. MPEP-induced rotations: Bars represent the mean (±S.E.M.) number of net rotations as recorded during 120 min of registration following injection with MPEP at doses of 7.5, 15 or 30 mg/kg, p.o. ($n = 12$ per treatment group), the vehicle (methylcellulose (0.5%), $n = 11$) or (+)MK-801 (0.3 mg/kg, i.p., $n = 11$). * = $P < 0.05$, *** = $P < 0.001$ vs. vehicle (0 mg/kg).

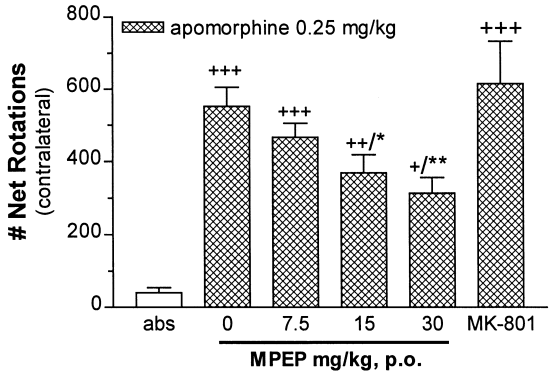

Fig. 2. Apomorphine-induced rotations: Bars represent the mean (±S.E.M.) number of net rotations in 90 min of registration following treatment with apomorphine (0.25 mg/kg, s.c.). The animals were pre-treated for 30 min with MPEP at doses of 7.5, 15 or 30 mg/kg, p.o. ($n=12$ per treatment group), the vehicle (0 mg/kg; methylcellulose (0.5%), $n=10$) or (+)MK-801 (0.3 mg/kg, i.p., $n=11$). An additional group of animals was included that was treated with vehicle only, i.e. the absolute control (abs; $n=7$). * = $P<0.05$, ** = $P<0.01$ vs. vehicle (0 mg/kg); + = $P<0.05$, ++ = $P<0.01$, +++ = $P<0.001$ vs. the absolute control (abs).

3.3.2. Combination MPEP and apomorphine

Apomorphine (0.25 mg/kg, s.c.) induced a significant increase ($P<0.001$) in the number of net rotations when compared to the absolute control. MPEP decreased the number of apomorphine-induced net rotations in a dose-dependent manner (ANOVA, $F=4.972$, $P<0.005$). While 7.5 mg/kg of MPEP was ineffective, 15 and 30 mg/kg significantly reduced the number of net rotations ($P<0.05$ and $P<0.01$, respectively; Fig. 2). (+)MK-801 (0.3 mg/kg, i.p.) induced no statistically significant changes in apomorphine-induced rotations (Fig. 2).

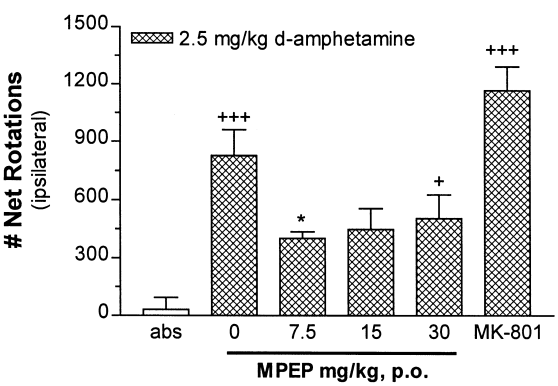

Fig. 3. D-Amphetamine-induced rotations: Bars represent the mean (±S.E.M.) number of net rotations in 180 min of registration following treatment with D-amphetamine (2.5 mg/kg, i.p.). The animals were either pre-treated for 30 min with MPEP at doses of 7.5, 15 or 30 mg/kg, p.o. ($n=15$ per treatment group), the vehicle (0 mg/kg; methylcellulose (0.5%), $n=15$) or (+)MK-801 (0.3 mg/kg, i.p., $n=15$). An additional group of animals was treated with vehicle only, i.e. the absolute control (abs; $n=12$). * = $P<0.05$ vs. vehicle (0 mg/kg); + = $P<0.05$, +++ = $P<0.001$ vs. the absolute control (abs).

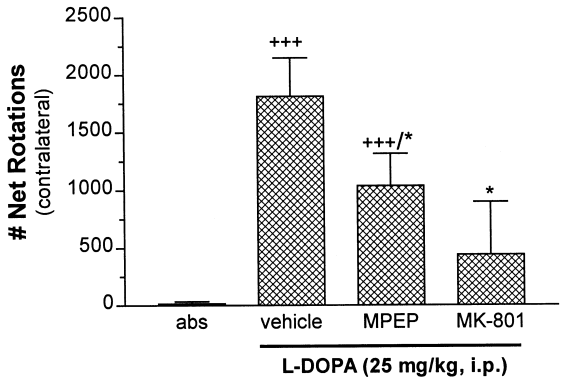

Fig. 4. L-DOPA-induced rotations: Bars represent the mean (±S.E.M.) number of net rotations in 240 min of registration following treatment with L-DOPA (25 mg/kg, i.p.). The animals were either pre-treated for 30 min with MPEP (30 mg/kg, p.o.; $n=15$ per treatment group), the vehicle (0 mg/kg; methylcellulose (0.5%), $n=13$) or (+) MK-801 (0.3 mg/kg, i.p., $n=15$). An additional group of animals was treated with vehicle only, i.e. the absolute control (abs; $n=5$). * = $P<0.05$ vs. vehicle (0 mg/kg); +++ = $P<0.001$ vs. the absolute control (abs).

3.3.3. Combination MPEP and D-amphetamine

D-Amphetamine (2.5 mg/kg, i.p.) induced a significant increase ($P<0.001$) in the number of net rotations when compared to the absolute control. MPEP decreased the number of D-amphetamine-induced rotations (ANOVA, $F=11.353$, $P<0.001$; Fig. 3). While the dose of 7.5 mg/kg significantly reduced the number of net rotations, the effects of higher doses, i.e. 15 and 30 mg/kg did not reach the level of significance. In a separate experiment, the effect of lower doses (1 and 3 mg/kg) of MPEP was investigated and it was found that both doses were ineffective although the dose of 3 mg/kg tended to decrease the D-amphetamine-induced rotations (data not shown). (+)MK-801 (0.3 mg/kg, i.p.) potentiated the D-amphetamine-induced rotations; however, this effect did not reach significance ($P=0.083$).

3.3.4. Combination MPEP and L-DOPA

L-DOPA (25 mg/kg, i.p.) induced a significant increase ($P<0.001$) in the number of net rotations when compared to the absolute control (ANOVA, $F=4.111$, $P<0.05$). MPEP (30 mg/kg, p.o.) and (+)MK-801 (0.3 mg/kg, i.p.) significantly reduced L-DOPA-induced rotations ($P<0.05$; Fig. 4).

4. Discussion

In the present study, four major findings were obtained. First, the rotarod experiments indicate that MPEP in the dose range between 7.5 and 300 mg/kg did not cause any deterioration of motor performance on the accelerating rotarod in rats. These data indicate that MPEP, even at fairly high doses of 100 mg/kg and up, and probably also other $mGlu_5$ receptor antagonists of the same chemical class, do not have serious deleterious effects on motor

behaviour in rats, such as ataxia or severe forms of sedation, rigidity or muscle relaxation.

Second, MPEP was found to inhibit spontaneous locomotor activity at doses of ≥ 30 mg/kg, i.e. at doses which induce turning behaviour in unilateral lesioned animals. Whether this effect reflects an influence on a clear motor component (however, see above) or an effect on premotor planning, is currently unknown and beyond the scope of the present study. Nevertheless, given the near maximal possible scores on the accelerating rotarod (see above), a non-motor component may be indicated.

Third, MPEP induced a dose-dependent ipsilateral rotational response in unilateral 6-OHDA lesioned rats. Although the effect was quite small, it was found consistently and has in the meantime been confirmed with other $mGlu_5$ receptor antagonists of the same chemical class (Spooren et al., unpublished observation). The fact that MPEP alone induced turning behaviour could favor the hypothesis of symptomatic benefit of $mGlu_5$ receptor antagonists in Parkinson's disease (see below). However, given the limited motor response following application of MPEP, these data may indicate that $mGlu_5$ receptor antagonists, if at all beneficial in Parkinson's disease (see below), may not be sufficiently effective to be used as a stand-alone symptomatic treatment to replace existing dopamine-based treatments. Furthermore, the finding that 30 mg/kg MPEP attenuated spontaneous locomotor activity in intact non-lesioned animals but increased activity in unilateral 6-OHDA-lesioned animal seems contradictive. Obviously, this underscores the need for a better understanding of the plasticity in this receptor in the basal forebrain and it may reflect the dynamics of $mGlu_5$ receptors in the diseased brain (see below).

Explaining the underlying mechanism of MPEP-induced rotations is mere speculation. However, it could be postulated that the effects induced by MPEP alone may reflect a direct interaction of dopamine and glutamate on striatal medium spiny neurons. As $mGlu_5$ receptors are highly expressed in striatal medium spiny neurons (Shigemoto et al., 1993; Romano et al., 1995), they may regulate the glutamatergic cortical-striatal input. Consequently, inhibition of this glutamatergic input is known to change the dopaminergic-glutamatergic balance in favor of dopamine (Amalric et al., 1994; Calabresi et al., 1997; Morari et al., 1998) and via such an action, MPEP may have induced the (weak) rotational response. Alternatively, inhibition of the excitatory subthalamic efferent pathways may equally well explain these findings. Clearly, the underlying mechanism remains to be further investigated in future studies.

Fourth, MPEP dose-dependently inhibited apomorphine-induced contralateral rotations. A straightforward subtraction of MPEP- and apomorphine-induced rotations cannot account for these findings: a dose of 15 mg/kg MPEP induced ± 20 ipsilateral rotations (90 min of registration) but MPEP inhibited ± 200 apomorphine-induced contralateral rotations (120 min of registration), i.e. approximately 10 times more. Given the fact that apomorphine mediates its effects post-synaptically and that both receptors, i.e. $mGlu_5$ and dopamine, are highly expressed in striatal medium spiny neurons, it is probable that these effects reflect an interaction of $mGlu_5$ and dopamine receptors on the level of the intra-cellular signaling pathways.

MPEP also inhibited D-amphetamine-induced ipsilateral rotations. Again, these data cannot be explained by a simple addition of their independent rotational effects since both compounds induce ipsilateral rotations. Although not completely without controversy (see: Hu et al., 1999), it has been suggested that the mixed groups I and II receptor agonist aminocyclopentane-1,3-dicarboxylic acid (ACPD) may increase dopamine release in freely moving animals (Bruton et al., 1999; cf. Bruton et al., 1996a,b). Further experiments indicated that especially the group I receptors may be responsible for this effect (Bruton et al., 1999). In addition, rotational behaviour is induced by direct injections of mixed groups I and II receptor agonists into the striatum (Smith and Beninger, 1996; also see: Sacaan et al., 1991, 1992). Taking these data together it could be hypothesized that the agonists of the $mGlu_5$ receptor may potentially increase dopamine release whereas $mGlu_5$ receptor antagonists in turn may inhibit dopamine release. Accordingly, MPEP may have inhibited D-amphetamine-induced dopamine release and causing a reduction in rotations. However, given the relative lack of $mGlu_5$ receptors on the pre-synaptic side, a post-synaptic mechanism, i.e. on the level of the intra-cellular signalling pathways as outlined above, may be more probable.

Finally, the effective dose of MPEP, i.e. 30 mg/kg, was chosen to evaluate its effect on L-DOPA-induced rotations. In line with the above discussed findings, MPEP significantly inhibited the L-DOPA-induced rotations. Again, a simple calculation of ipsilateral vs. contralateral rotations cannot explain the data; an interaction of the intra-cellular signalling pathways on the post-synaptic level may again seem likely (see discussion on apomorphine) although it is important to note that an interaction of different efferent pathways within or outside the basal ganglia can also explain these findings. Again mechanism of action awaits to be investigated in future studies.

The present findings indicate that any dopamine-mediated rotation response is inhibited by MPEP in the 6-OHDA lesioned rat. However, inhibition of dopamine-mediated behaviours is so far only seen in this lesion model since MPEP has been shown to have no effect on D-amphetamine-induced locomotor activity (Spooren et al., 2000) or on apomorphine-induced climbing in the intact mouse (Spooren et al., unpublished observation). Accordingly, these data suggest that in response to dopaminergic denervation the sensitivity of specific brain regions to mGluR5 antagonists may change markedly either within but also outside the affected brain regions.

The present study only partly confirmed the (+)MK-801-induced potentiation of dopamine-mediated responses or in the case of L-DOPA even opposite effects to those described in the literature were found (Carlsson and Carlsson, 1989; Klockgether and Turski, 1990; Morelli and Di Chiara, 1990; Morelli et al., 1992). It has to be noted that (+)MK-801 was used here as an effective reference compound and its interaction with either apomorphine or D-amphetamine was not per se the aim of investigation. Therefore, experimental conditions were not adapted according to anticipated results in a particular experiment but rather a fixed dose regimen was chosen. In the studies describing the potentiation of dopamine-mediated responses, the applied doses of (+)MK-801 were ineffective in inducing a rotational response by themselves (Carlsson and Carlsson, 1989; Klockgether and Turski, 1990; Morelli and Di Chiara, 1990; Morelli et al., 1992; Gossel et al., 1995). Since (very) low doses of (+)MK-801 potentiate dopamine-mediated responses in models of Parkinson's disease (Carlsson and Carlsson, 1989; Klockgether and Turski, 1990; Morelli and Di Chiara, 1990; Morelli et al., 1992), future studies might focus on effects of low-very low dosages of MPEP to indeed investigate a (+)MK-801-like action of MPEP.

It has been postulated that $mGlu_5$ receptors may be a novel drug target for the symptomatic treatment of Parkinson's disease (Nicoletti et al., 1997). Antagonists of mGlu receptors may reduce the activity of the overactive excitatory glutamatergic subthalamic-pallidal/nigral pathways that are thought to inhibit motor activity or provide symptomatic relief through other channels (Smith and Parent, 1988; Albin et al., 1989). Although the underlying mechanism is currently unknown, MPEP increased the number of net rotations as outlined above which may be an indication of treatment potential. Furthermore, MPEP has a relatively large safety margin since it did not induce any rotarod disturbance at doses of up to 300 mg/kg. However, a major drawback of $mGlu_5$ receptor antagonists for the treatment of Parkinson's disease is the fact that virtually any dopamine-mediated response in the 6-OHDA rat rotation model was shown to be inhibited by MPEP. In addition, MPEP at high doses inhibited spontaneous locomotor activity in rats. Accordingly, these data suggest that $mGlu_5$ receptor antagonists may counteract the symptomatic benefit provided by L-DOPA or by one of the direct dopamine receptor agonists currently on the market. Taking these present findings into consideration then it would become obvious that MPEP and potentially other $mGlu_5$ receptor antagonists are probably not appropriate drug candidates for the symptomatic treatment of Parkinson's disease.

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