0022-3565/10/3323-1064–1071$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 332:1064–1071, 2010

Vol. 332, No. 3
162115/3557377
Printed in U.S.A.

Metabotropic Glutamate 7 Receptor Subtype Modulates Motor Symptoms in Rodent Models of Parkinson's Disease

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Received September 28, 2009; accepted November 24, 2009

ABSTRACT

Metabotropic glutamate (mGlu) receptors modulate synaptic transmission in the central nervous system and represent promising therapeutic targets for symptomatic treatment of Parkinson's disease (PD). Among the eight mGlu receptor subtypes, mGlu7 receptor is prominently expressed in the basal ganglia, but its role in restoring motor function in animal models of PD is not known. The effects of *N*,*N*′-dibenzhydrylethane-1,2-diamine dihydrochloride (AMN082), the first selective allosteric activator of mGlu7 receptors, were thus tested in different rodent models of PD. Here, we show that oral (5 mg/kg) or intrastriatal administration (0.1 and 0.5 nmol) of AMN082 reverses haloperidol-induced catalepsy in rats. AMN082 (2.5 and 5 mg/kg) reduces apomorphine-induced rotations in unilateral 6-hydroxydopamine (6-OHDA)-lesioned rats. In a more complex task commonly used to evaluate major akinetic symptoms of PD patients, 5 mg/kg AMN082 reverses the increased reaction time to respond to a cue of bilateral 6-OHDA-lesioned rats. In addition, AMN082 reduces the duration of haloperidol-induced catalepsy in a mGlu7 receptor-dependent manner in wild-type but not mGlu7 receptor knockout mice. Higher doses of AMN082 (10 and 20 mg/kg p.o.) have no effect on the same models of PD. Overall these findings suggest that mGlu7 receptor activation can reverse motor dysfunction associated with reduced dopamine activity. Selective ligands of mGlu7 receptor subtypes may thus be considered as promising compounds for the development of antiparkinsonian therapeutic strategies.

Parkinson's disease (PD) is a chronic neurodegenerative disorder that results from the loss of dopaminergic neurons in the substantia nigra pars compacta and innervating the striatum. Dopamine replacement therapies provide years of symptomatic benefit in PD but often leads to the development of abnormal involuntary movements, such as dyskinesia, after long-term treatment. There is hope to find alternative therapeutic strategies that, despite the reduced dopamine activity, can reverse dysfunction of the motor circuit in PD patients. Among them, regulating glutamate overactive transmission in the basal ganglia via the modulation of the three groups of metabotropic glutamate (mGlu I, II, and III) receptors appears to be an attractive target. Clinical and preclinical evidence emphasizes the role of group I postsynaptic mGlu5 receptor subtype and group III presynaptic mGlu receptor subtypes (mGlu4, 6, 7, and 8 receptors) in reversing motor deficits in PD and dopamine-deficient animals (Ossowska et al., 2001; Breysse et al., 2002, 2003; Feeley Kearney and Albin, 2003; Conn et al., 2005; Lopez et al., 2007). In particular, within group III, nondiscriminative but nonetheless group III-selective mGlu receptor agonists including 1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-1; Acher et al., 1997) and L-(+)-2-amino-4-phosphonobutyric acid (L-AP4) can reverse reserpine or haloperidol-induced catalepsy and reduce motor symptoms in 6-OHDA-lesioned rats (Kearney et al., 1998; Zhang and Albin, 2000; Senkowska and Ossowska, 2003; Valenti et al., 2003; Lopez et al., 2007).

This work was supported by the Centre National de la Recherche and Aix-Marseille University; the 'Fondation de France Comité Parkinson'; National Research Agency [Grant ANR-05-NEUR-021-01]; and Era-net NEURON network [Grant ANR-08-NEUR-006-01]. B.G. was supported by the Fondation de France, and S.L. was supported by the Ministry of Education and Research and Foundation France Parkinson.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.109.162115.

ABBREVIATIONS: PD, Parkinson's disease; mGlu, metabotropic glutamate receptor; ACPT-1, 1-aminocyclopentane-1,3,4-tricarboxylic acid; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; 6-OHDA, 6-hydroxydopamine; AMN082, *N*,*N*′-dibenzhydrylethane-1,2-diamine dihydrochloride; KO, knockout; RT, reaction time; MSX-3, ((*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl]ester); WT, wild type; DA, dopamine; ANOVA, analysis of variance; PLSD, protected least significant difference; GP, globus pallidus.

Selective action on mGlu4 receptors may account for these positive results because recent orthosteric compounds, that better discriminate mGlu4 receptor subtype among group III mGlu receptors (Beurrier et al., 2009), or positive allosteric modulators (Marino et al., 2003; Battaglia et al., 2006; Niswender et al., 2008; Conn et al., 2009), were shown to have anticataleptic and neuroprotective properties in rodents, presumably by a selective action on the GABAergic striatopallidal pathway. In addition to mGlu4 receptor, the group III presynaptic receptor mGlu7 receptor is widely expressed in the basal ganglia neurocircuit. A role of mGlu7 receptors has largely remained unexplored because of the lack of mGlu7 receptor-specific reagents, while its high evolutionary conservation (Flor et al., 1997; Conn and Niswender, 2006) and abundance in the basal ganglia circuit suggest that it may display a fundamental role in the control of normal and abnormal motor activity (Kinoshita et al., 1998; Kosinski et al., 1999). The recent discovery of an orally active, brain-penetrable, and selective allosteric activator of mGlu7 receptors, AMN082 (Mitsukawa et al., 2005), prompted us to study the role of this receptor in dopamine-depleted rodents to assess its potential target for the treatment of motor dysfunction in PD.

Materials and Methods

Animals

Male Wistar rats (280–350 g; Charles River, l'Arbresle, France) were housed in groups of two per cage and maintained in temperature-controlled conditions with a 12-h light/dark cycle (7:00 AM—7:00 PM, lights off). In the reaction time task, male Wistar rats weighing 175 to 185 g at the beginning of the experiment were fed 15 to 17 g/day laboratory chow delivered 3 h after the testing period, so as to maintain 85% of their free feeding body weight. Water was provided ad libitum.

mGlu7 Receptor Knockout Mice

The generation of mGlu7 receptor knockout (mGlu7 KO) mice was described previously (Sansig et al., 2001). mGlu7 KO mice were generated from embryonic day 14 (129/Ola) stem cells. All of the mice used in the studies reported here carried either two wild-type or two mutant mGlu7 receptor alleles on a 14th generation (F_{14}) C57BL/6 genetic background. Male littermate mice were used in all experiments. Food pellets and tap water were available ad libitum. All animal behavioral and surgical procedures were conducted in accordance with the requirements of the European Communities Council (Directive 86/609/EEC, November 24, 1986).

Surgery

Unilateral 6-OHDA Nigral Lesions. Male Wistar rats (280–300 g; $n = 28$) were anesthetized with equithesin (9.7 mg/ml sodium pentobarbital in 0.9% NaCl + 42.6 mg/ml chloral hydrate in propylene glycol + 21.2 mg/ml Mg_2SO_4 in ethanol; 3.0 ml/kg i.p.) and received desipramine hydrochloride injection (25 mg/kg i.p.; Sigma-Aldrich, Lyon, France) 30 min before surgery, to protect noradrenergic cells. They were placed in a stereotaxic apparatus (David Kopf instruments, Tujunga, CA) with the incisor bar positioned −3.3 mm under the interaural line. 6-OHDA (2 μg/μl in ascorbate 0.1%) or vehicle solution (sham) was injected through 30-gauge stainless steel injector needles into the left or right substantia nigra pars compacta at the following coordinates (Paxinos and Watson, 2006): AP, −5.2 mm; L, ± 2.1 mm; and DV, −7.6 mm according to bregma. The flow rate (0.5 μl/min) and volume (4 μl) of injections were controlled with a micropump (CMA/100; CMA Microdialysis, Stockholm, Sweden) using a 10-μl Hamilton microsyringe (Hamilton Co., Reno, NV) connected by a Tygon catheter (0.25 mm i.d.) fitting to the 30-gauge stainless steel injector needles. Three additional minutes were allowed for diffusion of the toxin.

Bilateral 6-OHDA Striatal Lesions. Male Wistar rats ($n = 27$) previously trained in the reaction time (RT) task were anesthetized with a combination of xylazine (15 mg/kg) and ketamine (100 mg/kg i.p.). 6-OHDA hydrochloride (4 μg/μl; 3 μl/side) or vehicle solution was bilaterally injected in the striatum at the following coordinates: AP, −0.2 mm; L, ±3.5 mm; and DV, −4.8 mm according to bregma by using the same injection procedure.

Intrastriatal Injections. Xylazine/ketamine anesthetized male Wistar rats ($n = 21$) were implanted with 10-mm bilateral stainless steel guide cannulae (23-gauge) positioned 3 mm above the same striatal injection site. Guide cannulae were anchored to the skull with four stainless steel screws and dental cement. After a 7-day recovery period, intrastriatal injections were performed in the awake rats by gently inserting the injector needles (13 mm; 30 g) inside the guide cannulae, and they were fitted so that they protruded 3 mm below into the striatum. The flow delivered by the pump was set at 0.16 μl/min for a volume of 1 μl/side as described above.

Drugs. 6-OHDA and apomorphine (Sigma-Aldrich) were dissolved in 0.1% ascorbic acid solution. Haloperidol (1 mg/ml; Sigma-Aldrich) was prepared in distilled water with a methylparaben and propylparaben solution and a drop of lactic acid (0.1 N). Apomorphine and haloperidol were injected in a volume of 1 ml/kg. The mGlu7 receptor-positive allosteric modulator AMN082 (Novartis, Basel, Switzerland) was freshly suspended in 0.5% methylcellulose aqueous solution and injected by gavage (oral administration) in a volume of 10 ml/kg in mice and 5 ml/kg in rats. The selective A2A receptor antagonist MSX-3 (Sigma-Aldrich) (Müller et al., 1998) was dissolved in distilled water, pH 7, and administered intraperitoneally.

Behavioral Tests

Haloperidol-Induced Catalepsy. Male Wistar rats ($n = 30$) received 1 mg/kg haloperidol intraperitoneally followed 30 min later by AMN082 (0, 5, 10, and 20 mg/kg p.o.; $n = 7–8$/group). After 30 min, the latency (in seconds; cut-off time, 120 s) to step down a rod suspended 9 cm above the floor was measured every 30 min during the 2-h testing. Another group of haloperidol-injected rats received intrastriatal injections of AMN082 (0, 0.1, and 0.5 nmol/0.5 μl; $n = 6–8$/group) and were tested immediately afterward in the bar test every 10 min for 1 h. To assess the mGlu7 receptor-dependent action of AMN082, wild-type (WT) and mGlu7 KO mice treated with 6 mg/kg haloperidol were challenged to AMN082 administration (0, 1, and 6 mg/kg p.o.; $n = 7–10$/group), and the latency to step down a thin rod suspended 4 cm above the floor was measured at each 30-min point for 2 h. Considering the different sensitivity of mGlu7 KO mice to haloperidol treatment in comparison with WT mice, AMN082 effects were further tested in mGlu7 KO mice pretreated with 10 mg/kg haloperidol. AMN082 doses were chosen on the basis of previous results (Mitsukawa et al., 2005; Palucha et al., 2007). Moreover, we tested the effect of A2 adenosine receptor antagonist MSX-3 (2.5 mg/kg), known for its antiparkinsonian properties (Lopez et al., 2008; Salamone et al., 2008), in mGlu7 KO mice pretreated with 10 mg/kg haloperidol ($n = 6$).

Apomorphine-Induced Circling. Thirty days after surgery, unilateral 6-OHDA-lesioned rats received AMN082 administration (0, 2.5, 5, and 10 mg/kg p.o.; $n = 6–7$/group) or vehicle (0.5% methylcellulose) 30 min before apomorphine injection (0.2 mg/kg s.c.). Immediately afterward, they were placed in automated rotameter cylinders (TSE, Bad Homburg, Germany), and the number of ipsi- and contralateral rotations was recorded for 90 min.

Reaction Time Task. As described previously (Amalric et al., 1995), rats were trained daily in eight operant boxes (Campden Instruments Ltd., Cambridge, UK) that were placed in a wooden sound-attenuating cage equipped with a retractable lever, a food magazine, and a cue light (2.8-W bulb) above the lever. Pressing the lever required a force of 0.7 N for switch closure. Rats were trained

to quickly release the lever at the light-cue onset presented after four randomly and equiprobably generated foreperiods (0.5, 0.75, 1.00, and 1.25 s). To be rewarded by a food pellet (45 mg; Bioserve, Frenchtown, NJ), rats were required to release the lever with RTs below 600 ms. RTs were measured in milliseconds as the time elapsing from the response signal onset to the lever release. Performance was analyzed in terms of correct or incorrect: either delayed (lever release above 600 ms) or premature (lever release before the light-cue onset) unrewarded responses by session. Each daily session ended after 100 trials, excluding premature responses. After 2 to 3 months of training, baseline performance (85–90% correct responses) was measured for four consecutive sessions before surgery. After 7 day recovery, control and 6-OHDA-lesioned rats were tested for up to 19 days after surgery. On day 20, all rats received vehicle gavage to familiarize them to oral administration. Sham ($n = 12$) and 6-OHDA lesioned rats ($n = 15$) received four doses of AMN082 (0, 5, 10, and 20 mg/kg p.o.) in a pseudorandom order following a Latin-square design to test for the order of injections. Each animal received AMN082 or vehicle injection at day 21, 25, 30, and 34 after surgery.

Histology

At completion of the behavioral testing, rats were killed by decapitation and brains were stored at −80°C until cryostat sectioning. Coronal (10-μm) sections were collected (−20°C) at striatum level using a cryostat apparatus (model CM3050; Leica, Wetzlar, Germany). The extent of the lesions was verified by autoradiographic labeling of DA uptake sites with [^{3}H]mazindol in bilateral and unilateral 6-OHDA-lesioned groups. Binding of [^{3}H]mazindol was measured according to the procedure described previously (Javitch et al., 1985). In brief, sections were first air-dried and rinsed for 5 min at 4°C in 50 mM Tris buffer with 120 mM NaCl and 5 mM KCl. Then, the sections were incubated for 40 min with 15 nM [^{3}H]mazindol (PerkinElmer, Boston, MA; specific activity 17 Ci/mM) in 50 mM Tris buffer containing 300 mM NaCl and 5 mM KCl added with 0.3 mM desipramine to block noradrenaline transporter. Possible unspecific binding was determined by incubating some sections in the same solution plus 30 mM benztropine. Sections were rinsed twice for 3 min in the incubation medium without mazindol and for 10 s in distilled water and were air-dried. Autoradiograms were generated by opposing the sections to ^{3}H-sensitive screen (Raytest, Courbevoie, France) for 21 days and were further quantified with a Fuji-Bas 5000 beta imager (Fujifilm, Tokyo, Japan).

Statistical Analysis

Catalepsy. Median latency to step down the bar was analyzed with a nonparametric multiple Kruskal-Wallis H test. Individual comparisons between treatment groups and time periods were performed using the nonparametric Mann-Whitney U test.

Circling Behavior. The number of net rotations (i.e., number of contralateral minus ipsilateral rotations) induced by apomorphine was analyzed by repeated measures ANOVA with AMN082 doses as between-subject factor and time within-subject factor. Individual comparisons at distinct time points used unpaired t test.

Reaction Time Task. Pre- and post-6-OHDA lesion (days 12–15) performance was averaged across four sessions. Because there was no difference between days across "pre" and "post" conditions for each variable (number of correct, delayed, premature trials, and RT), mean pre and post performance was compared with that measured after AMN082 treatment. Data were submitted to a mixed design ANOVA with different groups (sham versus 6-OHDA) as the between-subject factor and conditions (pre, post, vehicle, and AMN082 doses) as the within-subject factor. Post hoc multiple comparisons between groups were made using simple main effects analysis and Fisher's PLSD test, as appropriate.

Results

AMN082 Effects on Haloperidol-Induced Catalepsy in Rats. Haloperidol (1 mg/kg) produced profound cataleptic effects in rats as shown by a dramatic increase in the latency to step down the rod (Fig. 1). AMN082 at a dose of 5 mg/kg ($P < 0.01$, Mann-Whitney U test; Fig. 1a) significantly reduced step-down latency as found previously with other non-subtypes selective group III agonists (Lopez et al., 2008). Significant reversal of haloperidol-induced catalepsy was achieved at time points 30, 60, and 90 min ($P < 0.01$, Mann-Whitney U test; Fig. 1b). AMN082 (10 mg/kg) tended to decrease haloperidol-induced catalepsy at the same intervals, whereas 20 mg/kg had no effect. Local infusion of AMN082 (0.1–0.5 nmol/0.5 μl) into the striatum significantly reversed catalepsy at the two doses tested ($P < 0.05$, Mann-Whitney U test; Fig. 2, a and b), suggesting that striatal mGlu7 receptors are involved in the antiparkinsonian action of AMN082.

AMN082 Effects on Apomorphine-Induced Circling. 6-OHDA infusion in the substantia nigra pars compacta resulted in a nearly complete loss of [^{3}H]mazindol binding in the ipsilateral striatum, with some remaining labeling into the nucleus accumbens (Fig. 3A). Quantification revealed 80 to 90% depletion in the whole striatum (dorsal and ventral), as found previously (Darbaky et al., 2003). In this model of dopamine depletion, AMN082 at doses of 2.5 and 5.0 mg/kg reduced apomorphine-induced contralateral rotations (significant dose × time interaction: $F_{15,120} = 1.99$, $P < 0.05$; Fig. 4). AMN082 (2.5 mg/kg) decreased the total number of 625 ± 86.6 net rotations per 90 min induced by apomorphine

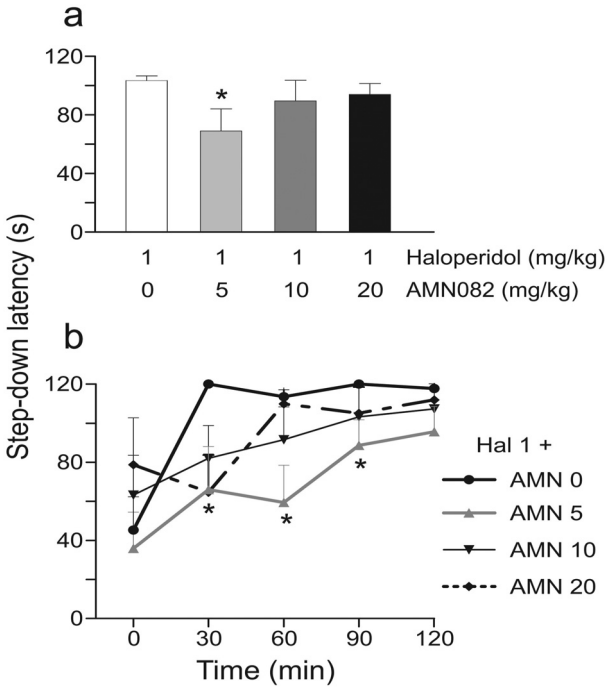

Fig. 1. Effects of AMN082 on haloperidol-induced catalepsy in rats. Rats were treated with 1 mg/kg i.p. haloperidol. After 30 min, animals received an oral administration of AMN082 (0, 5, 10, and 20 mg/kg; $n = 7–8$/group), and catalepsy was measured 30 min after injection for 120 min. The graphs represent the mean latency to step down the rod ± S.E.M. during the 120-min test (a) and at each time point (30 min) for 120 min (b), as described under *Materials and Methods*. ∗, $P < 0.05$, significant difference versus vehicle group (nonparametric Mann-Whitney U test).

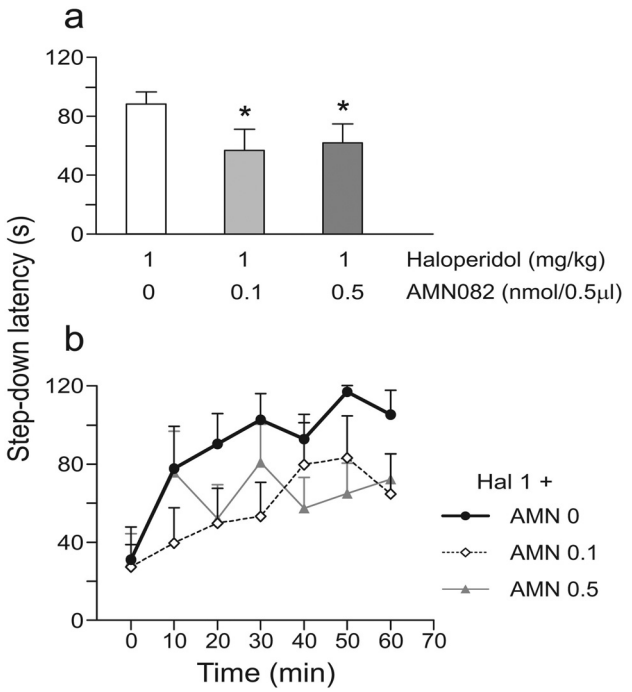

Fig. 2. Effects of intrastriatal infusion of AMN082 on catalepsy. Haloperidol injected rats (1 mg/kg i.p.) received bilateral infusion of AMN082 (0, 0.1, and 0.5 nmol/0.5 μl; $n = 6–8$/group) into the striatum and were tested in the catalepsy test every 10 min for 60 min. The graphs represent the mean latency to step down the rod ± S.E.M. during the 60-min test (a) and every 10 min for 60 min (b). ∗, $P < 0.05$, significant difference versus vehicle-treated rats (nonparametric Mann-Whitney U test).

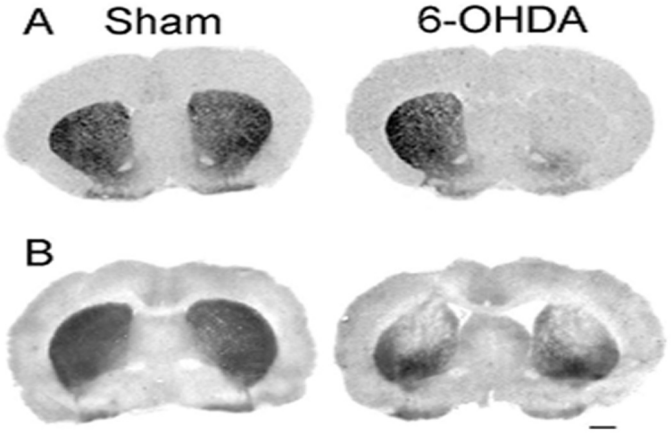

Fig. 3. Binding of [^{3}H]mazindol to dopamine uptake sites in the striatum. Photomicrographs comparing [^{3}H]mazindol labeling in striatal sections from a sham-operated animal (left) and a 6-OHDA-lesioned animal (right). A, representation of the typical unilateral 6-OHDA lesion tested in the circling behavior (AP, 0.84 mm related to bregma; Paxinos and Watson, 2006). B, typical bilateral 6-OHDA lesions restricted to the dorsal striatum (AP, 0.36 mm related to bregma; Paxinos and Watson, 2006) in animals tested in the reaction time task. Scale bar, 2 mm.

to 285 ± 100.8 (, $P < 0.05$, Fisher's PLSD test; Fig. 4a), with a significant effect during the first 45 min at the peak action of apomorphine (Fig. 4b). A similar tendency was observed at a dose of 5 mg/kg, whereas AMN082 at a dose of 10 mg/kg had no effect.

AMN082 Effects in the Reaction Time Task. Bilateral 6-OHDA infusion into the striatum produced partial DA depletion in rats that were restricted mainly to the dorsal part of the striatum compared with control animals (Fig. 3B). As

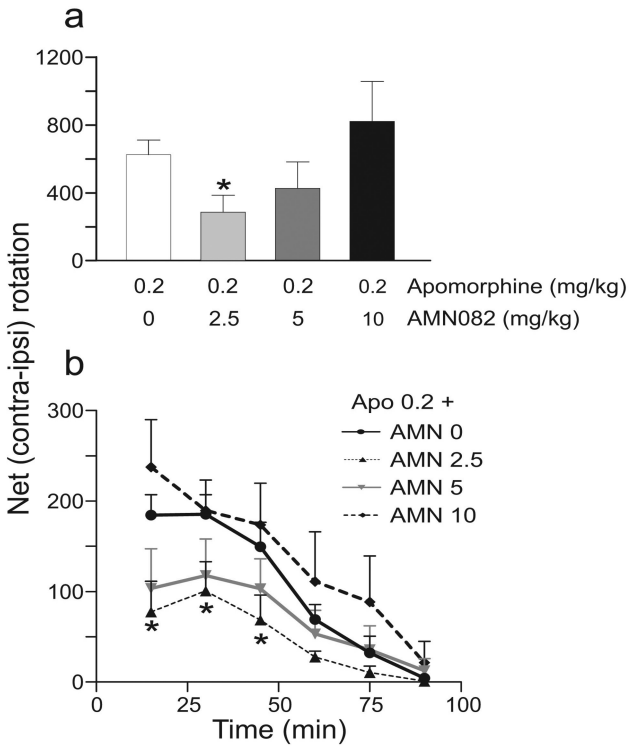

Fig. 4. Effects of AMN082 on apomorphine-induced circling behavior. Unilateral 6-OHDA-lesioned rats were treated with AMN082 (0, 2.5, 5, and 10 mg/kg p.o.; $n = 6–7$/group), and 30 min later they received apomorphine injection (0.2 mg/kg s.c.). The total number of ipsilateral and contralateral rotations was measured for 90 min immediately after apomorphine injection. The graphs represent the mean ± S.E.M. of net rotations (number of contralateral minus ipsilateral rotations) for 90 min (a) and every 15-min interval (b). ∗, $P < 0.05$ versus vehicle group (unpaired t test).

found previously, such 6-OHDA infusions typically induced 50 to 60% depletion of striatal DA levels, causing impairment of movement initiation (akinesia) (Amalric et al., 1995; Lopez et al., 2007). As illustrated in Fig. 5 and Table 1, the number of correct responses after 6-OHDA lesions was markedly reduced compared with preoperative levels or sham-operated animals (group × lesion interaction: $F_{1,21} = 10.9$, $P < 0.05$, Fisher's PLSD test; Table 1). There was no significant change in premature responding after lesion whatever the group ($P > 0.05$ N.S.; Table 1). In contrast, the number of delayed responses (over the 600-ms time limit) significantly increased compared with preoperative sessions ($P < 0.05$, paired t test) or with the sham group ($P < 0.05$, Fisher's PLSD test; Fig. 5b and Table 1). When plotting frequency of reaction time against reaction time intervals, 6-OHDA lesions decreased significantly the height of the RT-frequency distribution curves (Fig. 5c). Mean preoperative RTs, 349 ± 6.2 ms, were increased up to 379 ± 7.1 ms after 6-OHDA lesions (Fig. 5c). The onset of these changes was rapid (postoperative day 10), and baseline performance did not recover within the testing period.

AMN082 at doses of 5 and 10 mg/kg markedly reversed the lesion-induced increases of delayed responses (Fig. 5b) without altering performance of sham-operated animals (group × treatment interaction: $F_{5,105} = 6.94$, $P < 0.01$ and AMN082 treatment effect: $F_{5,105} = 14.64$, $P < 0.01$; Table 1). AMN082 at a dose of 5 mg/kg restored the shape of the RTs distribution curve compared with preoperative sessions (Fig. 5d). There was no effect related to the order of AMN082 doses

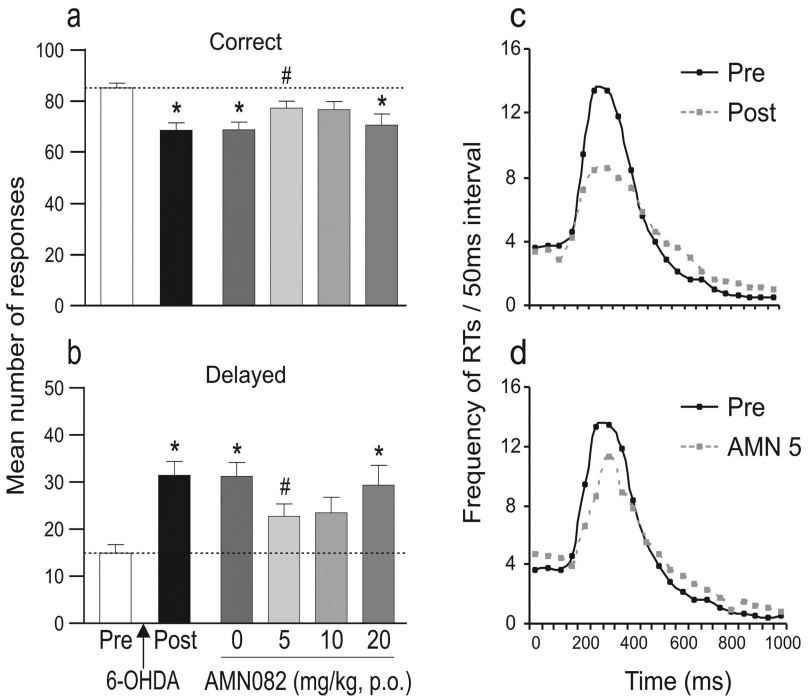

Fig. 5. a and b, effects of AMN082 in the reaction time task. Bilateral 6-OHDA-lesioned rats ($n = 15$) were injected with AMN082 (0, 5, 10, and 20 mg/kg, p.o.) after a pseudorandom Latin-square design, and the effects were measured 30 min later on the mean number of correct (a) and delayed (b) responses ± S.E.M. in the reaction time task. Performance was collapsed over four preoperative (pre) and four postoperative sessions (post), and AMN082 was injected at different doses at days 21, 25, 30, and 34 after surgery. c and d, effects of AMN082 5 mg/kg on RT distributions in 6-OHDA-lesioned rats. RT frequency (i.e., percentage) of RTs by 50-ms interval is plotted from 0 to 1000 ms after the visual cue onset. *, $P < 0.05$, significant difference versus preoperative level (Fisher's PLSD test after significant ANOVA); #, $P < 0.05$, significant difference versus postoperative level (Fisher's PLSD test after significant ANOVA).

TABLE 1
Effects of AMN082 (5, 10, and 20 mg/kg) on correct, delayed, and premature responses in 6-OHDA-lesioned and sham-operated rats

	Pre	Post	0	5	10	20
				mg/kg		
6-OHDA-lesioned rats						
Correct	85.1 ± 1.8	68.6 ± 3.0*	68.8 ± 3.0*	77.3 ± 2.7#	76.5 ± 3.2	70.7 ± 4.2*
Delayed	14.9 ± 1.8	31.4 ± 3.0*	31.2 ± 3.0*	22.7 ± 2.7#	23.5 ± 3.2	29.3 ± 4.2*
Premature	44.3 ± 6.1	48.0 ± 7.5	50.5 ± 11.6	65.7 ± 12.1	61.9 ± 13.7	61.7 ± 11.9
Sham-operated rats						
Correct	85.1 ± 1.7	84.1 ± 1.4	84.1 ± 2.4	83.6 ± 3.2	81.6 ± 2.4	61.2 ± 5.1*#
Delayed	14.9 ± 1.7	15.9 ± 1.4	15.9 ± 2.4	16.4 ± 3.2	18.4 ± 2.4	38.8 ± 5.1*#
Premature	43.4 ± 4.6	34.5 ± 3.9	44.3 ± 13.2	40.1 ± 5.1	42.9 ± 10.5	25.1 ± 4.3

* $P < 0.05$ versus pre (Fisher's PLSD test).
# $P < 0.05$ versus post (Fisher's PLSD test).

used in the Latin-square design in line with the stability of the treatment-induced action on behavior over time. Post hoc comparisons revealed a significant reduction of delayed responses in the 6-OHDA-lesioned rats treated with 5 mg/kg AMN082 and a nearly significant trend at a 10-mg/kg dose ($P = 0.06$) compared with their postoperative performance or after vehicle treatment ($P < 0.05$, Fisher's PLSD test). A dose of 20 mg/kg AMN082 had no more beneficial effects in 6-OHDA-lesioned rats and increased delayed responding and RTs in sham animals ($P < 0.05$, Fisher's PLSD test after significant ANOVA $F_{5,45} = 12.31$; Table 1). These effects reflect a slowing in the task execution despite the fact that AMN082 did not alter spontaneous motor activity (data not shown).

AMN082 Effects on Haloperidol-Induced Catalepsy in Wild-Type and mGlu7 Receptor Knockout Mice. mGlu7 KO mice differed from WT mice in their cataleptic response to haloperidol administration (Fig. 6a). Catalepsy was achieved after 6 mg/kg haloperidol in both groups, although to a lesser extent in mGlu7 KO mice ($P < 0.05$, Mann-Whitney U test). AMN082 at a dose of 1 mg/kg significantly reduced haloperidol-induced catalepsy in wild type ($P < 0.05$, Mann-Whitney U test) and had no effect in mGlu7 KO mice (Fig. 6, b and c). At a 6 mg/kg dose, AMN082 showed a trend to reduce catalepsy in both groups, but this effect did not reach significant level. To further characterize AMN082 action on a similar level of catalepsy, a group of mGlu7 KO mice received a dose of 10 mg/kg haloperidol. In this condition, AMN082 did not reverse haloperidol-induced catalepsy at any dose (Fig. 6d), whereas treatment with the A2 adenosine receptor antagonist MSX-3 in mGlu7 KO mice was found to have anticataleptic properties in the same test (Fig. 6e).

Discussion

The results of the present study demonstrate that the mGlu7 receptor selective allosteric activator AMN082 can reduce haloperidol-induced catalepsy and reverse 6-OHDA-induced nigrostriatal dopamine depletion-related behavioral deficits in rats. At efficacious doses, AMN082 reverses haloperidol-induced catalepsy in wild-type but not mGlu7 knockout mice. Using mGlu7 receptor knockout and wild-type mice, mGlu7 receptor-dependent effects of AMN082 were also found on plasma corticosterone and adrenocorticotropin levels and anxiolytic behavior (Mitsukawa et al., 2005; Sta-

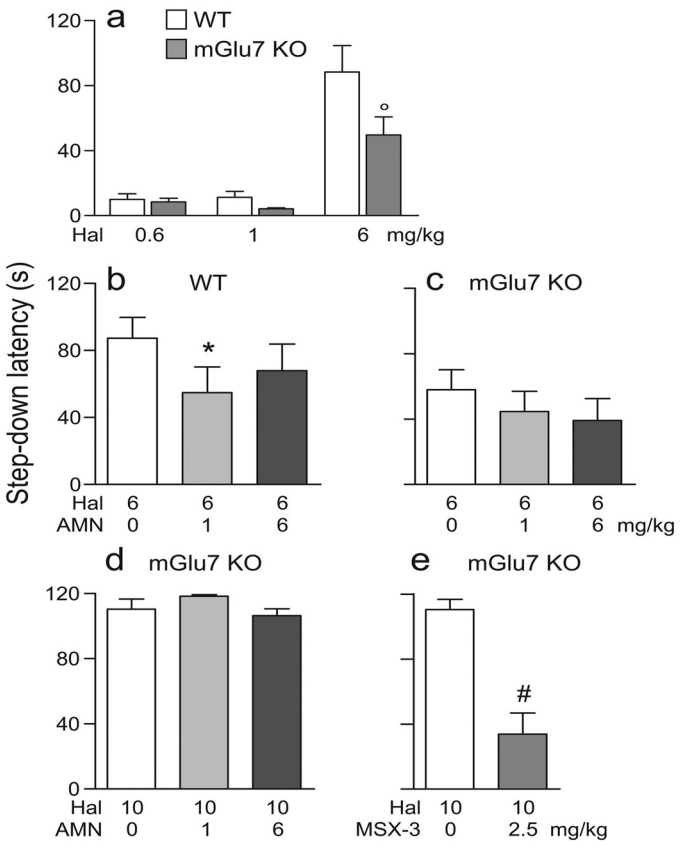

Fig. 6. Effects of AMN082 on catalepsy in wild-type and mGlu7 receptor knockout mice. The graphs represent the mean latency ± S.E.M. to step down the rod of WT and mGlu7 KO A2A mice after various doses of haloperidol (0.6, 1, and 6 mg/kg i.p.; a) and after oral administration of AMN082 (0, 1, and 6 mg/kg p.o; n = 7–10/group; b–d). AMN082 (1 mg/kg) significantly decreased the latency to step down the rod in WT mice (b) but not in mGlu7 KO mice pretreated with 6 mg/kg haloperidol (c) or 10 mg/kg haloperidol (d). The adenosine receptor antagonist MSX-3 (2.5 mg/kg) significantly decreased the latency to step down the rod in mGlu7 KO mice pretreated with 10 mg/kg haloperidol (e). °, $P < 0.05$, significant difference from WT mice. ∗, $P < 0.05$, significant difference from vehicle-injected WT mice and #, $P < 0.05$, significant difference from vehicle-injected mGlu7 KO mice (Mann-Whitney U test).

chowicz et al., 2008). These findings demonstrate that the anticataleptic effects of AMN082 are mediated by mGlu7 receptors.

The beneficial effects of AMN082 in rats were evident in the various behavioral tests at a narrow dose range (2.5–5 mg/kg), lost efficacy at 10 mg/kg, and started to cause behavioral impairment at 20 mg/kg in control animals. Several possible explanations may account for a bimodal activity of AMN082. 1) A preferential action of AMN082 at high doses in the substantia nigra pars reticulata may occur. Presynaptic activation of group III mGlu receptors on GABA nerve terminals in the substantia nigra, with ACPT-1, L-AP4 (Lopez et al., 2007), or AMN082 (B. Greco and M. Amalric, unpublished data), increases RTs and induces catalepsy in control animals. Therefore, as suggested by Albin et al. (1989), a reduction of GABAergic activity in the substantia nigra pars reticulata reinforces thalamic inhibition of the sensorimotor cortex and ultimately may account for these motor disabilities. 2) A direct inhibitory effect on nigrostriatal DA transmission leading to motor impairment may occur. L-AP4, injected at high doses presumably activating mGlu7 receptors, decreases DA extracellular content in the dorsal striatum and the nucleus accumbens and reduces psychostimulant-induced hyperactivity in rats (Hu et al., 1999; Mao et al., 2000; Mao and Wang, 2000). 3) Effects at the highest doses are off-target and independent of mGlu7 receptors. AMN082, at comparable doses to those used in the present study, decreases locomotor activity of mGlu7 receptor knockout and wild-type mice, demonstrating mGlu7 receptor-unrelated effects (Palucha et al., 2007; Salling et al., 2008). In line with these complex effects of AMN082 in vivo, recent in vitro studies reported a lack of action of AMN082 in reducing synaptic transmission at the Schaffer collateral-CA1 synapse, although mGlu7 receptor probably plays a predominant action in regulating transmission at that level in adult rats (Ayala et al., 2008). Furthermore, depending on the exact type of cellular assay and/or cellular background, AMN082 may loose its efficacy in activating G protein-mediated intracellular signaling (Suzuki et al., 2007; Ayala et al., 2008). 4) Finally, the internalization and loss of mGlu7 receptor cell surface expression (Pelkey et al., 2007) after administration of AMN082 could account for a bimodal effect of AMN082. High but not low doses of AMN082 may favor internalization and result in different behaviors.

The selectivity of AMN082 on mGlu7 receptors may be predominant, however, if low concentrations are used as illustrated by its positive effect in different rodent models of PD. AMN082 decreased rotational behavior induced by apomorphine in rats with unilateral 6-OHDA-induced lesions of the nigrostriatal dopamine pathway, a standard paradigm for testing the efficacy of antiparkinsonian treatments that may offer symptomatic relief in later stages of PD (Ungerstedt, 1971). For example, deep brain stimulation of the subthalamic nucleus, well recognized to have beneficial effects in the treatment of PD, counteracts apomorphine-induced rotations in unilateral 6-OHDA-lesioned rats and reduces catalepsy induced by haloperidol (Darbaky et al., 2003). In line with this, intrapallidal injection of ACPD, an mGlu receptor nonsubtype-selective agonist, decreases amphetamine-induced rotations (Agari et al., 2008). Furthermore, the group III nonsubtype-selective agonists L-AP4 and ACPT-1 reverse reserpine or haloperidol-induced catalepsy and reduce motor asymmetries in unilateral 6-OHDA-lesioned rats (Marino et al., 2003; Valenti et al., 2003; MacInnes et al., 2004; Konieczny et al., 2007; Lopez et al., 2007, 2008). Our results indicate that the antiparkinsonian actions of these agents may in part be attributed to the activation of mGlu7 receptors. These simple sensorimotor tests used in lesioned rodents have commonly been used to validate experimental therapies that may reverse motor deficits in PD. They also provide valid tests to search for mechanisms counteracting effects caused by dopamine depletion but independent of actions on dopamine receptors. Movement deficits in PD patients are, however, associated with more complex changes in the motor control neural network than those occurring in most pharmacologically induced animal models that may be assessed with RT behavioral procedures (Amalric et al., 1995; Gauntlett-Gilbert and Brown, 1998). Here, we used an RT task in rats to assess whether AMN082 can reverse deficits also in the initiation of movement (akinesia), a cardinal motor deficit in PD. In rats with partial bilateral 6-OHDA nigrostriatal lesions, a state that is more representative of early degenerative stages in PD, AMN082 at doses of 5 and 10 mg/kg reduced the number of delayed responses

and decreased RTs. These results support a critical role for allosteric activation of mGlu7 receptor to improve the motor and nonmotor symptoms of PD as recently found with allosteric modulation of mGlu5 receptors in the same paradigm or on muscular rigidity (Ossowska et al., 2001; Breysse et al., 2002, 2003).

The beneficial effects produced by mGlu7 receptor activation may preferentially involve non-DA mechanisms. In parkinsonian conditions, the degeneration of DA neurons is associated with major disruption of the glutamate and GABA function in the basal ganglia circuitry (Albin et al., 1989). Accordingly, presynaptic activation of group III mGlu receptor subtypes in the globus pallidus (GP) with nonsubtype-selective group III agonists (L-AP4, L-serine-*O*-phosphate, or ACPT-1) reduce haloperidol, reserpine or 6-OHDA-induced motor impairment, presumably by reducing GABAergic activity (Valenti et al., 2003; MacInnes et al., 2004; Konieczny et al., 2007; Lopez et al., 2007; Sibille et al., 2007; Agari et al., 2008). A preferential action on mGlu4 rather than seven receptor subtypes in the GP is suggested by others, and our preliminary observations show no beneficial effects of intrapallidal AMN082 injections on haloperidol-induced catalepsy (B. Greco and M. Amalric, unpublished data). The low level of mGlu7 receptor expression in the GP could account for this lack of effect. In contrast mGlu7 receptors are highly expressed in the striatum and the substantia nigra (Bradley et al., 1999; Kosinski et al., 1999; Messenger et al., 2002). In the striatum, mGlu7 receptors are localized presynaptically on the corticostriatal glutamatergic synapses, where they are found to decrease the glutamatergic tone and attenuate transmission in cholinergic interneurons (Pisani et al., 1997; Bell et al., 2002; Bonsi et al., 2008). Activation of group III mGlu receptors with ACPT-1 into the striatum produces antiparkinsonian effects by reducing catalepsy and striatal preproenkephalin mRNA levels induced by haloperidol (Konieczny et al., 2007). In line with these results, we found that the intrastriatal injections of AMN082 reverse haloperidol-induced catalepsy, suggesting that these antiparkinsonian effects could be mediated by the presynaptic activation of mGlu7 receptors on the glutamatergic corticostriatal synapses. mGlu7 receptors are primary localized in the active zone of the glutamatergic synapse and activation of these receptors with AMN082 would reduce the relatively high concentrations of glutamate in the corticostriatal synapses found in parkinsonian conditions.

Conclusions

Our findings suggest that under conditions of dopamine hypoactivity, as in PD, allosteric activation of mGlu7 receptors can significantly improve the basal ganglia motor neural network dysfunction. We provide the first evidence that mGlu7 receptor-selective activation could have potential for restoring motor deficits caused by dopamine depletion such as in PD and further supporting the hypothesis that mGlu receptor allosteric modulation may provide therapeutic benefit in neurological disorders (Conn et al., 2009). This study, however, also demonstrates a need for second generation mGlu7 receptor allosteric activators that can be safely administered over a wide dose range.

Acknowledgments

We thank Nathalie Turle-Lorenzo for technical assistance and Rémy Pernaud for computer programming.

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