Antiparkinsonian action of a selective group III mGlu receptor agonist is associated with reversal of subthalamonigral overactivity

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ABSTRACT

Activation of group III metabotropic glutamate (mGlu) receptors has been recently highlighted as a potential approach in the treatment of Parkinson's disease (PD). This study evaluates the antiparkinsonian action of systemic administration of the broad-spectrum agonist of group III mGlu receptors, 1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-I), and investigates its site of action within the basal ganglia circuitry. Acute injection of ACPT-I reverses haloperidol-induced catalepsy, an index of akinesia in rodents. In a rat model of early PD based on partial bilateral nigrostriatal lesions, chronic (2 weeks) administration of ACPT-I is required to efficiently alleviate the akinetic deficit evidenced in a reaction time task. This treatment counteracts the post-lesional increases in the gene expression of cytochrome oxidase subunit I, a metabolic marker of neuronal activity, in the overall subthalamic nucleus and in the lateral motor part of the substantia nigra pars reticulata (SNr) but has no effect in the globus pallidus. Paradoxically, ACPT-I administration in sham animals impairs performance and induces overexpression of cytochrome oxidase subunit I mRNA in the lateral SNr, and has no effect in the subthalamic nucleus or globus pallidus.

Altogether, our results provide new evidence for the antiparkinsonian efficiency of group III mGlu receptor agonism, point to the regulation of the overactive subthalamo-nigral connection as a main site of action in an early stage of PD and underline the complex interplay between these receptors and the dopaminergic system to regulate basal ganglia function in control and PD conditions.

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Introduction

The basal ganglia (BG) are a network of subcortical structures involved in the regulation of voluntary movements and cognitive processes. Dopamine (DA) and glutamate systems in the BG are oppositely regulated and play a crucial role in the pathophysiology of these structures. The degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) in Parkinson's disease (PD) results in

excessive activity of glutamatergic neurons of the subthalamic nucleus (STN) projecting to basal ganglia output structures, and this is believed to contribute to many of the motor and cognitive symptoms of PD (Wichmann and DeLong, 1996). The classical palliative therapy of PD with L-3,4-dihydroxyphenylalanine (L-DOPA) provides motor benefits but leads to motor complication after 5–10 years of treatment. Therefore, novel pharmacological treatments that specifically target this excessive glutamatergic activity may prove to be clinically beneficial. Because of their modulatory role in glutamatergic transmission, metabotropic glutamate (mGlu) receptors represent promising therapeutic targets for PD. The three groups of mGlu receptors are expressed in the BG where they regulate synaptic transmission and plasticity in physiological and pathological conditions (Conn et al., 2005). In particular, group III mGlu receptor subtypes (mGlu4, 7 and 8) are found in several structures within the BG, including the STN, striatum, substantia nigra pars compacta and pars reticulata (SNr), the external and internal segments of the globus pallidus (rodent's GP and entopeduncular nucleus, respectively) (Duty, 2010; Messenger et al., 2002; Testa et al., 1994). Group III mGlu receptors are expressed presynaptically and negatively coupled to adenylyl cyclase thereby decreasing excitability and neurotransmitter release. Direct injection of broad spectrum group III

agonists into the GP reverses akinesia produced by 6-OHDA lesions, haloperidol injections (Konieczny et al., 2007; Lopez et al., 2007) or reserpine treatment (MacInnes et al., 2004; Marino et al., 2003). Evidence points to a major role of the mGlu4 receptor subtype in mediating the antiparkinsonian effects in the GP (Beurrier et al., 2009; Marino et al., 2003). The effects produced by local activation of group III mGlu receptors in the SNr are more contrasted, as we previously found either alleviation or worsening of akinesia depending on the model of PD used (Lopez et al., 2007), thus questioning the efficacy of systemic treatment in models of PD. To clarify this issue, and because the systemic route of administration of antiparkinsonian compounds offers real therapeutic perspective, the first aim of this study was to evaluate the potential antiparkinsonian action of the group III-selective mGlu receptor agonist, 1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-I) in a 6-OHDA model of PD after intraperitoneal administration. The sites of action in the BG for the beneficial effects of group III mGlu receptor agonists in PD models being still uncertain, the second aim of the study was thus to assess the modulation of intraneuronal gene expression of cytochrome oxidase subunit I (CoI), a metabolic index of neuronal activity, in the GP, STN and SNr after ACPT-I chronic treatment for 2 weeks and to correlate these variations with the behavioral parameters.

Materials and methods

Animals

Male Wistar rats (n = 73, 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 am–7 pm, lights off). In the reaction time task, male Wistar rats weighing 175–185 g at the beginning of the experiment were fed 15–17 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*. All animal behavioral and surgical procedures were conducted in accordance with the requirements of the European Communities council directive n.86/609/EEC, November 24th, 1986.

Bilateral dopaminergic lesion

Animals (n = 38) previously trained in the RT task were anesthetized with a combination of xylazine (15 mg/kg) and ketamine (100 mg/kg i.p.) and placed in a stereotaxic apparatus (David Kopf instrument) with the incisor bar positioned −3.3 mm under the interaural line. 6-OHDA hydrochloride (4 μg/μl, 3 μl per side) or vehicle solution (sham) was bilaterally injected in the striatum at the following coordinates (Paxinos and Watson, 2005): AP −0.2 mm, L ±3.5 mm, DV −4.8 mm according to bregma. The flow rate (0.5 μl/min) and volume of injections were controlled with a micropump (CMA/100; CMA/Microdialysis, Stockholm, Sweden) using a 10 μl Hamilton microsyringe connected by a catheter (Tygon, i.d. 0.25 mm) fitting to the 30 gauge stainless steel injector needles. Three additional minutes were allowed for diffusion of the toxin.

Drugs

6-OHDA hydrochloride (Sigma Aldrich, Lyon, France) was dissolved in 0.1% ascorbic acid solution. Haloperidol (1 mg/ml, Sigma Aldrich, France) was prepared in distilled water with a methylparaben and propylparaben solution and a drop of lactic acid (0.1 N). Haloperidol was injected in a volume of 1 ml/kg (i.p.). The selective group III metabotropic glutamate receptor agonist (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT-I; Acher et al., 1997; Goudet et al., 2007; a generous gift from F. Acher, CNRS UMR 8601, Paris, France) was prepared freshly at the beginning of each experimental day. Initially, a 1 M solution of ACPT-I was made in 1.5 M NaOH, and then diluted 1:1 in sterile saline. Aliquots of the initial solution were

frozen at −80 °C, and further dilutions were then made in 0.9% sterile saline to obtain 30 and 10 mg/ml solutions.

Behavioral tests

Haloperidol-induced catalepsy

Five groups of male Wistar rats (n = 35, 280–350 g) were used (n = 7 per group). Three groups received ACPT-I at 1, 15 or 30 mg/kg; i.p., followed 30 min later by 1 mg/kg of haloperidol i.p. Two additional control groups received ACPT-I vehicle plus haloperidol or its vehicle. 60 min after haloperidol or vehicle injection, the latency (in seconds, cut-off time 120 s) to step down from a rod suspended 9 cm above the floor was measured every 10 min during the 1-hour testing.

Reaction time task

As described elsewhere (Amalric et al., 1995), rats were daily trained in eight operant boxes (Campden Instruments, Cambridge, UK), placed in a wooden sound-attenuating cage and 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, or 1.25 s). To be rewarded by a food pellet (45 mg, Bioserve, USA), rats were required to release the lever with RTs below 600 ms. RTs were measured in ms 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 2–3 months training, baseline performance (85–90% correct responses) was measured for 7 consecutive sessions before surgery. After 7 days of recovery, control (n = 7) and 6-OHDA lesioned rats (n = 6) were tested for up to 15 days post-surgery to assess postoperative performances. ACPT-I (10 or 30 mg/kg, n = 6–7 and n = 7–5 for sham and lesioned rats respectively) or vehicle was administered chronically for 2 weeks (day 16 to day 29, injections were made in between 8:30 and 10 am), and the animals were tested 1 h after injection. This timing was chosen according to blood–brain barrier penetration data showing that peak micromolar concentrations are found in the brain 1–2 h after drug administration (Lopez et al., 2008).

Tissue preparation and histological control of 6-OHDA lesion

The last experimental day (day 29), animals were killed by decapitation 30 min after the behavioral task (i.e. 90 min after the last ACPT-I/vehicle injection) and brains stored at −80 °C until cryostat sectioning. All the animals were killed during a 90-min time window (10 am to 11:30 am), in order to fit with the daily-drug injection cycle (8:30 am to 10 am), and to maximize homogeneity in respect to circadian cycle.

Coronal 10 μm sections were collected at striatum, GP, STN and SNr levels using a cryostat apparatus (Leica CM3050) at −20 °C. The sections were then mounted on SuperFrost Plus glass slides (Fisher Scientific) and stored at −80 °C until specific treatment. The extent of the 6-OHDA-induced dopamine denervation was examined at striatal level by autoradiographic labeling of [3H]-mazindol binding to DA striatal uptake sites, as described previously (Breysse et al., 2003; Oueslati et al., 2005). Briefly, 10 μm brain sections were air-dried and rinsed for 5 min in 50 mm Tris buffer with 120 mM NaCl and 5 mM KCl. They were then incubated for 40 min with 15 nM [3H]-mazindol (PerkinElmer; specific activity, 15–30 Ci/mmol) in 50 mm Tris buffer containing 300 mM NaCl and 5 mM KCl added with 0.3 mM desipramine to block the noradrenaline uptake sites. Sections were rinsed twice for 3 min in the Tris incubation buffer and for 10 s in distilled water and were air dried. Autoradiograms were generated by apposing the sections to [3H] sensitive X-ray films (Kodak Biomax

MR; Eastman Kodak, Rochester, NY, USA) for 28 days. Labeling was further quantified by digitized image analysis from film autoradiograms using the Densirag software from BIOCOM. Grey levels were converted to OD using external standards (calibrated density step tablet; Kodak). The mean OD value was determined from at least three sections per animal after subtracting the background signal measured on each section by scanning the cortex areas that are known to lack dopamine content.

In situ hybridization

Quantitative radioactive *in situ* hybridization was used to assess changes in intraneuronal mRNA levels of CoI, as a metabolic marker of neuronal activity. Oligoprobes (43–48 mer) were 3′-endlabeled by terminal deoxynucleotide transferase with 35S-dATP (1300 Ci/mmol). The radiolabeled probes were then purified on a mini Quick Spin Oligo Column (Roche, Meylan, France). All solutions used for *in situ* hybridization were treated with diethyl pyrocarbonate and autoclaved to avoid degradation by RNases. Slide-mounted sections were postfixed for 5 min in 3% paraformaldehyde and then incubated for 30 min in prehybridization buffer containing 2× standard saline citrate (SSC) and 1× Denhardt's solution. The sections were then acetylated for 10 min with 0.25% acetic anhydride in 0.1 M triethanolamine. Then, the tissue was treated for 30 min with 0.1 M Tris–glycine before being dehydrated in ethanol and air dried. Each section was covered with 35 µl of hybridization solution (4× SSC with 50% formamide, 10% dextran sulfate, 1× Denhardt's solution, 0.25 mg/ml *Escherichia coli* tRNA, and 0.5 mg/ml sheared salmon sperm DNA) containing the radiolabeled probe (radioactivity level ~400,000 cpm per section), and incubated overnight at 47 °C in humid chambers. After hybridization, the sections were then rapidly rinsed in ice-cold 2× SSC, then treated successively for 10 and 40 min with 1× SSC at room temperature, 1× SSC at 42 °C, and 0.1× SSC at 42 °C. Sections were then dehydrated in ethanol and air dried. Brain sections from each structure were then apposed to Kodak Bio-Max MR-1 film, and exposure time was adjusted to avoid film saturation. Sections processed for cellular analysis of COI mRNA levels were thereafter coated with LM1 autoradiographic emulsion (GE Healthcare, Piscataway, NJ), and exposed at 4 °C for 3 weeks. Exposed slides were developed in Kodak D-19 for 4 min at 13 °C and counterstained with toluidine blue.

Data analysis

Analysis of CoI mRNA labeling was performed at the cellular level on emulsion-coated sections at pallidal (−0.6 to −0.9 mm from bregma according to Paxinos and Watson, 2005), subthalamic (−3.6 to −4.16) and nigral levels (−5 to −5.4). In order to assess possible specific regional changes in CoI mRNA expression within each structure, subregions were delineated following functional territories. For the GP and the SNr, analysis was done on a lateral, central and a medial region corresponding to sensorimotor, associative and limbic territories (Deniau et al., 2007; Hamani et al., 2004; Joel and Weiner, 1997, 2000). In order to quantify a representative number of neurons (see details below), the analysis of the STN was performed on two regions, corresponding to the lateral and the medial part of the structure. Sections were observed under dark-field epilumination with a 20× immersion objective of a microscope connected to a COHU camera, and the digitized images were transferred to the screen of a video monitor with a resulting magnification of 1000×. Using the Visioscan image analysis software from BIOCOM, each neuron contour was drawn under bright field, and the number of silver grains per cell was estimated under polarized light by measuring optical density (OD) with respect to a standard curve of a defined number of silver grains. In the present experiments, since the autoradiographic background was very low, the corresponding value was not subtracted. A random sampling of at least 80–100 neurons per region (40–50 neurons per side, more than 10 grains/neuron) was quantified in two sections from each animal, and the mean number of silver grains per neuron was determined.

Statistical analysis

Catalepsy

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

Reaction time task

Pre- and post-6-OHDA lesion (days 9 to 15) performance was averaged across seven sessions. Since 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 to that measured after ACPT-I treatment. Data were submitted to a mixed design ANOVA with different groups ("Sham" vs "6-OHDA") as the between-subject factor and conditions (Pre, Post, vehicle, ACPT-I 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.

In situ hybridization

For CoI mRNA expression, the number of silver grain per neuron from all of the animals per condition were averaged and expressed as percentage ± standard error to the mean (SEM) of the corresponding control values. Statistical analyses were performed using a two-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference for multiple group comparison. A significance of $p < 0.05$ was required for rejection of the null hypothesis.

Results

Acute ACPT-I treatment reduces haloperidol-induced catalepsy

The statistical analysis revealed a significant effect of the different treatments ($p < 0.01$, Kruskal–Wallis test, H = 121.12). As illustrated in Fig. 1, animals treated with haloperidol alone reach the maximal cataleptic score at the second time point and remain cataleptic for the whole duration of the test ($p < 0.01$, Mann–Whitney U tests for "halo" vs "control" (NaCl), performed at each time point separately). Acute systemic administration of ACPT-I 25 mg/kg significantly reduces haloperidol-induced catalepsy whereas doses of 1 and 15 mg/kg do not produce any change. ACPT-I 25 mg/kg significantly reduces the step-down latencies at each time point for 40 min ($p < 0.05$, significantly
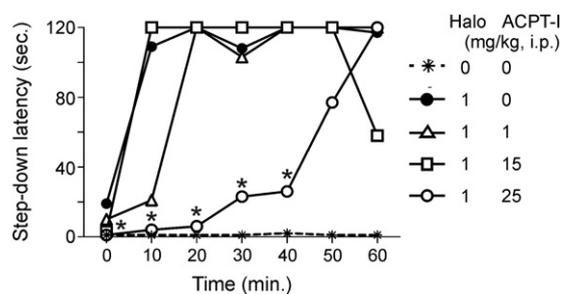
Fig. 1. Systemic injections of ACPT-I reverse haloperidol-induced catalepsy. Effect of systemic ACPT-I injections (0, 1, 15, 25 mg/kg i.p., n = 7 per group) on the median latency to step down from the rod for the 1 h test period. The groups received ACPT-I followed 30 min later by haloperidol 1 mg/kg i.p. and were tested 60 min later every 10 min for the whole duration of the test. The control group (n = 7) was injected with vehicle solutions. #Significantly different from control ($p < 0.05$; significant Mann–Whitney U test). *Significantly different from haloperidol group ($p < 0.05$; significant Mann–Whitney U test).
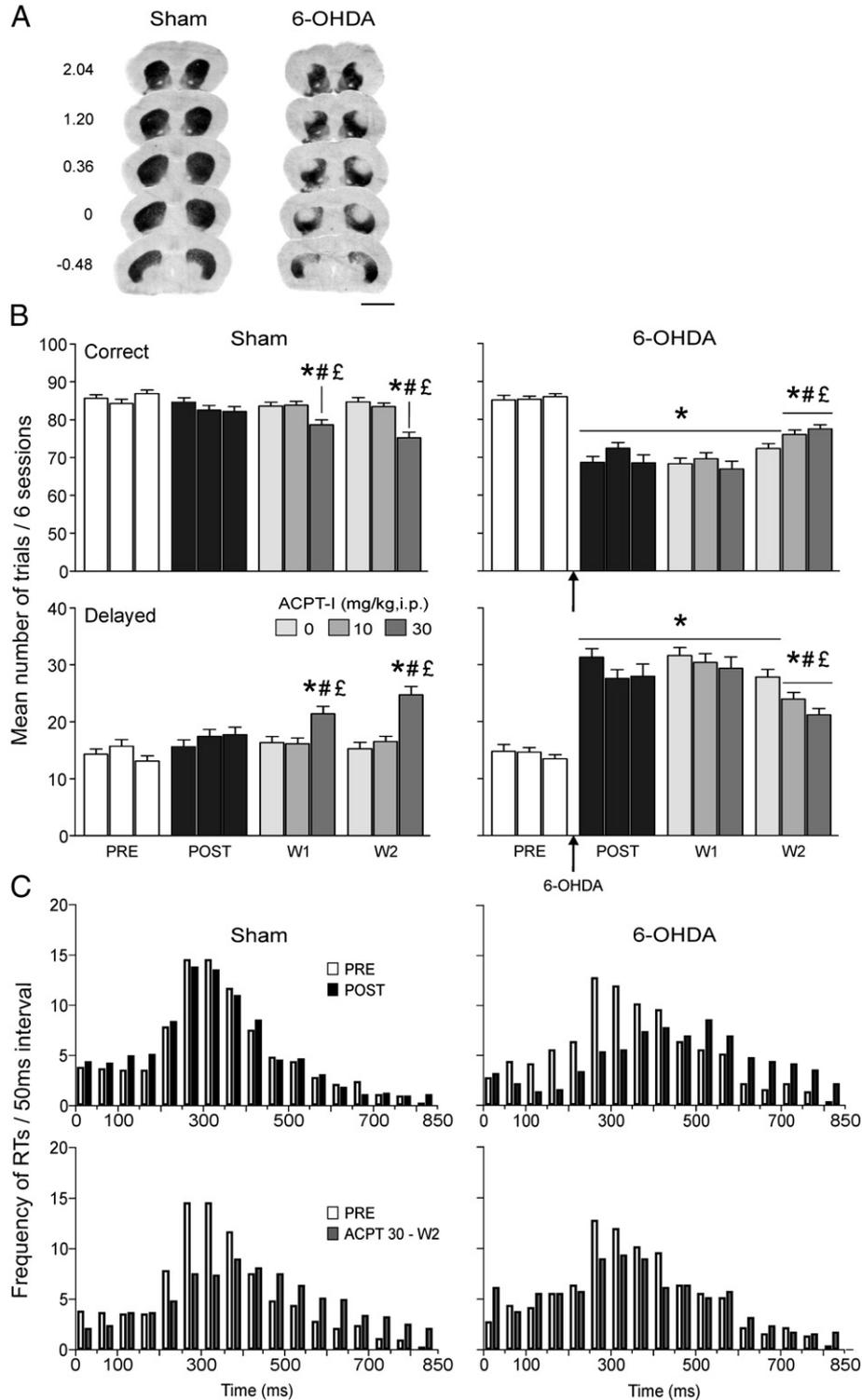
Fig. 2. Behavioral effects of chronic ACPT-I treatment in 6-OHDA lesioned animals on RT performance. A. Binding of [³H]-mazindol to dopamine uptake sites at the striatum level. Photomicrographs comparing the level of [³H]-mazindol labeling in striatal sections from representative subjects of the sham and 6-OHDA groups. The lack of mazindol binding shows the extent of the dopamine depletion in the dorsal striatum at five different anteriority levels [AP: from 2.04 to −0.48 mm related to bregma (Paxinos and Watson, 2005)]. Scale bar: 2 mm. B. Effects of chronic ACPT-I treatment on correct and delayed performance in control animals and after 6-OHDA lesion. Performance is expressed by the mean number of correct and delayed trials ± SEM, and measured on different blocks of seven sessions corresponding to the following: one block preceding the surgery (Pre), one block after the surgery from day 9 to 15 (Post), and two blocks during the ACPT-I treatment from days 16–21 (week1) and 22–29 (week 2). The effects induced by the different doses of ACPT-I (10 and 30 mg/kg) are compared with the preoperative, postoperative and vehicle performances. *Significantly different from preoperative performance (p<0.05; Fisher's PLSD test after significant ANOVA). #Significantly different from postoperative performance (p<0.05; Fisher's PLSD test after significant ANOVA). £Significantly different from vehicle level (p<0.05; Fisher's PLSD test after significant ANOVA). C. Effect of ACPT-I 30 mg/kg on RT distributions in control and 6-OHDA-lesioned rats. Each bar represents the mean number of RTs by 50-ms interval, plotted from 0 to 850 ms after the visual cue onset. The figure shows RT distributions during a prelesion (open bars), postlesion (black bars) and after ACPT-I chronic treatment (grey bars) session. 6-OHDA lesions produce a shift towards long RT values and ACPT-I treatment reverses this tendency at the second week of treatment, while the same ACPT-I treatment in sham-operated animals increases RTs.

different from the "halo" group, Mann–Whitney U tests), and loses its efficacy afterwards as seen by a return to maximal cataleptic level. ACPT-I did not produce catalepsy when tested alone (data not shown).

Partial bilateral 6-OHDA lesions impair movement initiation in the RT task

Bilateral infusion of 6-OHDA in the striatum produces a loss of DA nerve terminals restricted to its dorso-lateral part, but extending to the whole rostrocaudal axis of the striatum, as shown by decreased binding of [³H] mazindol to dopamine uptake sites (Fig. 2A). As reported previously with the same lesion procedure, 6-OHDA infusions induce a 60% decrease in [³H]-mazindol labeling in the dorsal area of the striatum compared with sham-operated animals (Turle-Lorenzo et al., 2006), which is in line with the decrease of endogenous striatal DA contents assessed by HPLC (Amalric et al., 1995).

The effects of the partial nigrostriatal 6-OHDA lesion were assessed by measuring RT performances as previously described (Lopez et al., 2007). Performance of sham animals remains unchanged after surgery (no significant difference between "Pre" vs "Post"; Fig. 2B). In contrast, 6-OHDA groups show a decrease of the mean number of correct responses mirrored by an increase of the mean number of delayed responses when compared to preoperative performances (Fig. 2B; $p < 0.01$, "Pre" vs "Post", Fisher's PLSD tests after a significant "time" effect, ANOVA, $F_{3,45} = 46.95$, $p < 0.01$ for correct and delayed responses). The deficit is confirmed by the shift to the right (towards the higher values) of the RT distribution curve (Fig. 2C). The onset of these changes is rapid (postoperative day 10) with no recovery of baseline performance within the testing period. 6-OHDA lesions do not modify the level of premature responses (data not shown).

Chronic ACPT-I treatment reduces 6-OHDA-induced akinesia

As shown in Fig. 2B, vehicle solution does not modify the level of performances, therefore showing that the 6-OHDA-induced deficits are stable throughout the experiment ($p < 0.05$, "Pre" vs "W1" and "Pre" vs "W2", PLSD tests). Whatever the group (sham or 6-OHDA-lesioned animals), acute administration of ACPT-I at the first day of the chronic treatment does not modify RT performance (data not shown). In contrast, after 2 weeks of daily injection, ACPT-I at the two doses tested (10 and 30 mg/kg, i.p.) reduces 6-OHDA-induced motor deficits. At the second week of treatment, a clear improvement of the performance is shown by a decreased number of delayed responses when compared to postoperative level ($p < 0.05$ and $p < 0.01$, "Post" vs "W2" for ACPT-I 10 and 30 mg/kg respectively, Fisher's PLSD tests) and to the control group ($p < 0.05$ and $p < 0.01$, during W2 for 10 and 30 mg/kg compared to vehicle, Fisher's PLSD tests after a significant ANOVA, $F_{(2,\ 123)} = 4.41$, $p < 0.05$). A two-week treatment with ACPT-I does not induce a full recovery, as correct and delayed responses remain significantly different from preoperative levels ($p < 0.01$, "Pre" vs "W2", Fisher's PLSD tests for correct and delayed responses). At the second week of treatment, however, the curve of RT frequency is shifted to the left, close to the distribution curve observed during preoperative period (Fig. 2C). Altogether, these results converge to show that a two-week treatment with the agonist of group III mGlu receptors reduces 6-OHDA-induced motor deficit in the RT task.

Chronic ACPT-I treatment disrupts performance of control animals

Interestingly, in sham-lesioned animals, the highest dose of ACPT-I produces opposite effects (Fig. 2B). After 1 week, and for the whole duration of the experiment, animals treated with the highest dose of ACPT-I (30 mg/kg) show a significant decrease in the number of correct responses, with a concomitant increase in the number of delayed responses, compared to pre and postoperative level ($p < 0.01$ and

$p < 0.05$, "Pre" vs "W1" and "Post" vs "W1" respectively; $p < 0.01$, "Pre" vs "W2" and "Post" vs "W2", Fisher's PLSD tests after a significant "time" effect, ANOVA, $F_{3,48} = 4.56$, $p < 0.01$ for correct and delayed responses). ACPT-I 30 mg/kg also impairs the RT distribution curve (Fig. 2C). After ACPT-I treatment, the curve show increased frequencies for longer RTs, similar to the pattern observed after 6-OHDA lesion.

Thus, systemic activation of mGlu receptors produces diametrically opposed effects in sham and 6-OHDA animals. Whereas it worsens the performances in sham, the same treatment, at a lower dose, alleviates 6-OHDA-induced motor deficits.

Chronic ACPT-I treatment differentially regulates metabolic activity in control and DA depleted animals

At completion of the behavioral test, the effects of bilateral 6-OHDA lesions and/or chronic ACPT-I treatment were determined on the level of CoI mRNA expression in lateral to medial subregions of the GP, STN and SNr and were related to the performances in the RT.

At the GP level and whatever the subregion examined, the expression of CoI mRNA is modified neither by the bilateral 6-OHDA lesions, nor by the ACPT-I treatment (Supplementary Fig. 1).

At the STN level (Fig. 3), the partial 6-OHDA lesions increase CoI mRNA expression in both the medial and the lateral parts of the nuclei when compared to control (+33% and +34%, $p < 0.05$ and $p < 0.01$ compared to sham; Fisher's PLSD test after a significant ANOVA, $F_{3,19} = 3.77$ and $F_{3,19} = 5.37$, $p < 0.05$ and $p < 0.01$ for medial and lateral part of the STN respectively). Chronic ACPT-I administration at the dose of 30 mg/kg that reduces 6-OHDA-induced motor impairment, normalizes levels of CoI mRNA in the lateral and medial parts of the STN ($p < 0.05$ compared to 6-OHDA; Fisher's PLSD test after a significant ANOVA), without modifying this parameter in control animals.

At the SNr level (Fig. 4), partial 6-OHDA lesions induce a drastic increase of CoI expression at the most lateral part of the structure (+44%, $p < 0.01$ compared to sham; Fisher's PLSD test after a significant ANOVA, $F_{3,19} = 7.38$, $p < 0.01$ for lateral SNr respectively), with a close to significant increase in the central part of the SNr (+27%, ANOVA, $F_{3,19} = 2.59$, $p = 0.08$). No modification of CoI mRNA expression was found at a more ventral level of the SNr. As shown in Fig. 4, chronic ACPT-I treatment reduces the lesion-induced abnormal expression of CoI in the lateral part ($p < 0.05$ compared to 6-OHDA; Fisher's PLSD test after a significant ANOVA) and the central part of the SNr. Interestingly, in line with the behavioral results showing a slowdown in movement initiation in sham animals treated with ACPT-I 30 mg/kg, the quantitative analysis of CoI expression shows a region specific increase in the lateral part of SNr only ($p < 0.05$ compared to sham; Fisher's PLSD test after a significant two-way ANOVA, Fig. 4).

These results are confirmed by the analysis of the frequency distribution of labeling per neuron. Indeed, at the level of lateral STN and SNr, the 6-OHDA-induced increased expression of CoI is illustrated by the shift to the right of the frequency distribution of labeling per cell (Fig. 5). The distribution of labeling per neuron shows that ACPT-I normalizes the distribution in 6-OHDA-lesioned animals at the lateral STN level. At the lateral SNr level, ACPT-I produces a slight improvement in 6-OHDA-lesioned rats whereas it produces a shift toward the right of the distribution in sham animals (Fig. 5).

Moreover, we examined the possible relationship between the cellular and the behavioral changes in 6-OHDA-lesioned animals. A positive correlation is found between the changes in CoI mRNA expression in the lateral parts of the STN or SNr and the RT performance after 6-OHDA lesion and ACPT-I 30 mg/kg administration ($R^2 = 0.479$, $p < 0.05$ and $R^2 = 0.659$, $p < 0.01$, for the STN and SNr respectively, Fig. 6).

Discussion

This study shows that systemic administration of the broad spectrum group III mGlu receptor agonist ACPT-I efficiently counteracts

Subthalamic nucleus

Fig. 3. Chronic treatment with ACPT-I reverses the DA lesion-induced increase in CoI gene expression in the STN. Upper panel shows representative level of STN [AP: −3.96 mm related to bregma (Paxinos and Watson, 2005)] illustrating the mediolateral delineation. Histograms represent the mean number of silver grains per labeled neuron in each region and in each experimental condition expressed as percentage ± SEM of sham animals treated with vehicle solution (n = 5–6 animals per group). *Significantly different from the sham group (p<0.05; Fisher's PLSD test after significant ANOVA). #Significantly different from 6-OHDA-lesioned animals treated with vehicle solution (p<0.05; Fisher's PLSD test after significant ANOVA).

Substantia nigra pars reticulata

Fig. 4. Chronic treatment with ACPT-I reduces the DA lesion-induced increase in CoI gene expression in the lateral SNr. Upper panel shows representative level of SNr (AP: −5.16 mm related to bregma; Paxinos and Watson, 2005) illustrating the medio-lateral delineation. Histograms represent the mean number of silver grains per labeled neuron in each region and in each experimental condition expressed as percentage ± SEM of sham animals treated with vehicle solution (n = 5–6 animals per group). *Significantly different from the sham group (p<0.05; Fisher's PLSD test after significant ANOVA). #Significantly different from 6-OHDA-lesioned animals treated with vehicle solution (p<0.05; Fisher's PLSD test after significant ANOVA).

the motor deficits induced by pharmacological or lesional alteration of dopamine transmission and that the anti-akinetic action in the lesion model is associated with reversal of neuronal metabolic changes in the STN and SNr. It extends the previous observation of a positive correlation between akinesia and the levels of CoI mRNA in the STN and SNr in the same model (Breysse et al., 2003; Oueslati et al., 2005) by showing that these changes in SNr are restricted to the lateral region of the SNr which receives motor striatal input, whereas they cover the different functional territories of the STN. Moreover, we evidenced a paradoxical akinetic action of ACPT-I treatment in control animals, accompanied by a selective increase of CoI expression in the lateral part of the SNr in the absence of change in the STN. These data suggest that the outcome of group III mGluR activation depends on dopamine tone and point to the subthalamic nucleus as a main site of the beneficial anti-akinetic action of ACPT-I.

Although several in vitro and vivo studies have highlighted the antiparkinsonian potential of group III mGlu receptor agonists (Duty, 2010), the effects of their systemic administration have been poorly investigated and mostly in non-lesional models of PD (Marino et al., 2003; Niswender and Conn, 2010). Our results are the first to demonstrate that a systemic administration of group III mGlu receptor agonist alleviates motor deficits after partial bilateral partial DA depletion in the striatum and to suggest that the subthalamonigral pathway is a main site for this beneficial action in the early stages of PD.

The selective group III mGlu receptor agonist ACPT-I was chosen because it shows similar affinity for mGlu4 and mGlu8 receptors than the classical agonist L-AP4, with higher concentrations required

to activate mGlu7, and was recently found to cross the blood brain barrier (Goudet et al., 2007; Lopez et al., 2008). Recent results show that agonists with preferential action at mGlu4 receptor subtype reduce parkinsonian symptoms (Beurrier et al., 2009; Marino et al., 2003; Niswender and Conn, 2010). This receptor subtype may thus represent the best candidate to explain the antiparkinsonian effects observed in the present study. The mGlu7 receptors may not be responsible of the ACPT-I effects as the doses used are not sufficient to deliver brain concentrations able to activate this subtype (Goudet et al., 2007; Lopez et al., 2008). Moreover, the selective activation of mGlu7 receptors in hippocampal neurons leads to a rapid internalization of this receptor (Pelkey et al., 2007), whereas stimulation of mGlu4 subtypes does not induce desensitization or internalization of the receptors in HEK cells (Mathiesen and Ramirez, 2006), thus
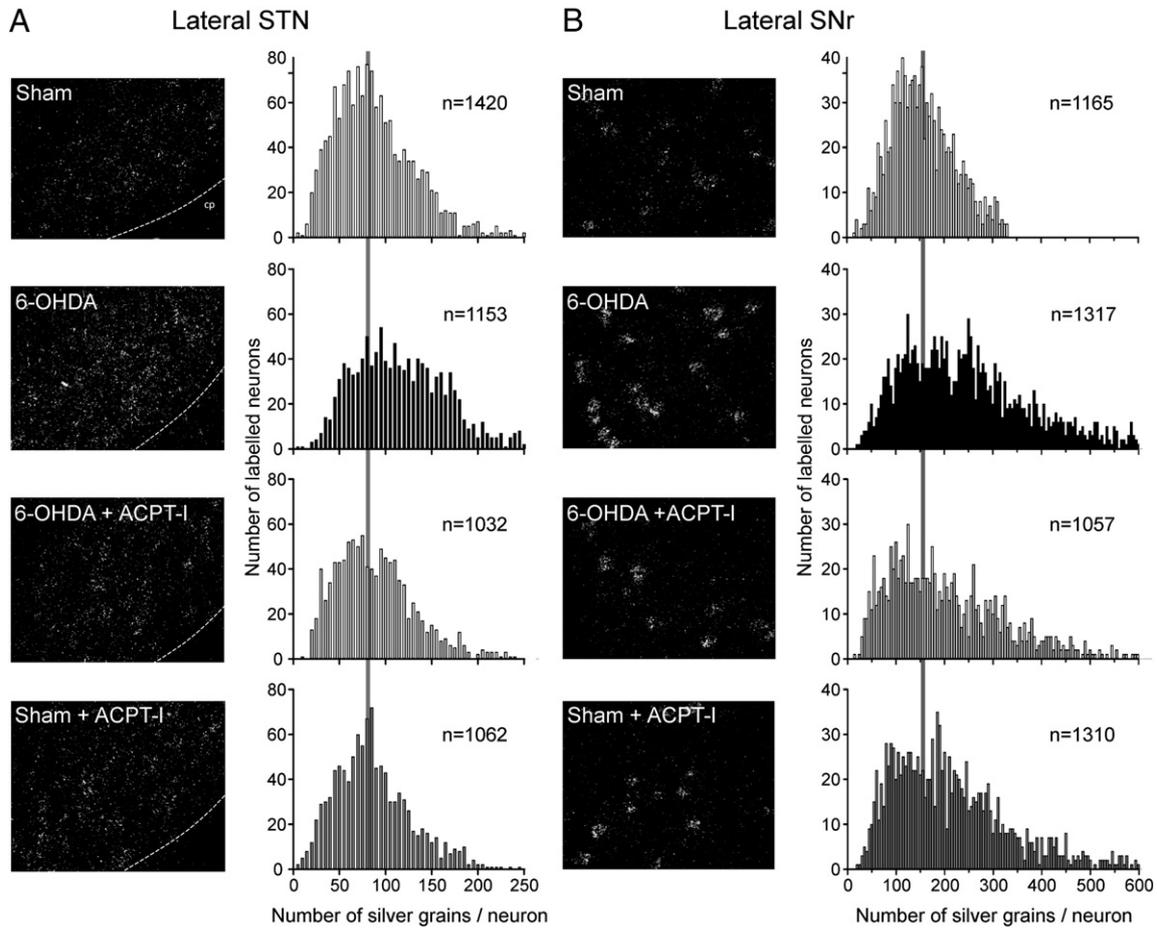
Fig. 5. Effects of 6-OHDA lesion and chronic treatment with ACPT-I on the distribution of labeling of COI mRNA in the lateral STN and SNr. Histograms of frequency distribution of labeling, show the effects of the dopamine lesion without or with subsequent treatment with ACPT-I 30 mg/kg, in the lateral STN (A) and the lateral SNr (B), and corresponding photomicrographs taken under dark-field epi-illumination. The vertical bar shows the median number of silver grains/neurons for the sham animals treated with vehicle solution. The lesion induces an increase in intraneuronal labeling as compared with controls, confirmed by a shift to the right of the distribution curve (toward the higher levels) in the lateral STN and SNr, that is reduced after ACPT-I treatment at 30 mg/kg. In control animals, ACPT-I increases intraneuronal labeling in the lateral SNr compared to the vehicle treated group.

arguing for a preferential action on mGlu4 versus mGlu7 subtype after chronic treatment. It is more likely that mGlu8 receptors contribute to the proakinetic effect of systemic administration of ACPT-I observed in control animals than to the beneficial action in 6-OHDA-lesioned rats. Indeed, intra-SNr injection of a preferential mGlu8 agonist (S)-DCPG was found to produce akinesia in control animals (Lopez et al., 2007), and even potentiate the akinetic deficit produced by the partial 6-OHDA lesion (unpublished personal observation).

Our results shed light on the BG subcircuits involved in the action of group III mGlu receptor agonists when administered systemically. Although reversing SNr overactivity, direct action of ACPT-I at basal ganglia output structure level to alleviate PD symptoms can be excluded, given that local administration of this compound in the SNr exacerbates parkinsonian deficits (Lopez et al., 2007). It is generally assumed that group III agonists may exert their positive action by reducing overactive striatopallidal GABAergic activity (Conn et al., 2005; Duty, 2010; Ossowska et al., 2007). This hypothesis is supported by the presynaptic expression of group III mGluRs at the striatopallidal synapses (Bradley et al., 1999; Corti et al., 2002; Galvan et al., 2006; Kosinski et al., 1999), where they regulate GABAergic inhibitory transmission (Macinnes and Duty, 2008; Marino et al., 2003; Matsui and Kita, 2003; Valenti et al., 2003). Considering the canonical model of BG anatomo-physiological organization (Albin et al., 1989; DeLong, 1990), disinhibiting GP neurons may increase their inhibitory tone on STN and ultimately reduce activity of the output structures of the BG. Accordingly, intrapallidal injection of different group III agonists,

including ACPT-I, was previously reported to counteract the parkinsonian-like motor deficits. However, our present data show that ACPT-I treatment did not modify the level of CoI mRNA in the GP of 6-OHDA animals while normalizing this marker in STN and SNr. These data suggest that systemic group III mGlu receptor agonists may produce their antiparkinsonian effects by acting primarily at the STN rather than at the GP level, by decreasing overactive subthalamonigral pathway activity, at least in the early PD stages. To our knowledge, little is known about direct effects of group III mGlu receptor agonists in the STN. ACPT-I treatment may provide beneficial motor effects in 6-OHDA animals by reducing the glutamatergic cortical or thalamic inputs to the STN. In line with this, L-AP4 reduces excitatory postsynaptic currents in the STN, by a presynaptic mechanism (Awad-Granko and Conn, 2001).

In contrast to its antiparkinsonian action in the 6-OHDA model of PD, ACPT-I produces akinesia in control rats. Whereas the akinetic deficits produced both by ACPT-I in control animals and by nigrostriatal 6-OHDA lesions seem similar, the underlying mechanisms appear to be different. The levels of CoI expression are enhanced in the SNr in both conditions, but CoI in the STN is unaffected in ACPT-I treated sham animals while being increased in the 6-OHDA lesioned animals. Thus, the motor deficits observed in control animals are associated with a specific change in the SNr whereas parkinsonian akinesia depends on overactive subthalamonigral pathway. Similarly, the difference between acute or delayed onset of systemic ACPT-I action in the haloperidol versus 6-OHDA conditions may rely on different sites of action in the BG. In the haloperidol model, local acute ACPT-I administration in either the GP or the SNr produces anticataleptic
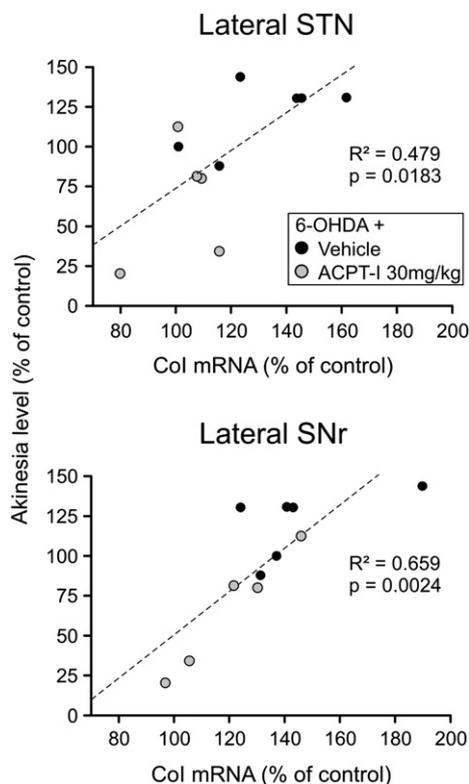
Fig. 6. Correlations between akinetic deficits and changes of CoI mRNA gene expression in the lateral parts of the STN and the SNr. The akinesia level is given, for each subject, by the mean number of delayed responses measured after the two-week ACPT-I treatment expressed as a percentage of control values (i.e. delayed responses before ACPT-I; postoperative period). Each dot represents the percent motor deficits after ACPT-I treatment with respect to percentage of change of CoI mRNA levels in individual animals from the different experimental groups. The decrease of CoI mRNA expression in the lateral parts of the STN and the SNr is correlated with a recovery of control performance (or reduced akinesia).

effects, whereas in the 6-OHDA model, opposite effects on akinesia are observed after GP or SNr administration (Konieczny et al., 2007; Lopez et al., 2007). This may account for the delayed action of ACPT-I in 6-OHDA model.

The present study, by showing differential behavioral and cellular effects in control and 6-OHDA lesioned rats, thus points to the dopaminergic system as a critical actor in the regulation of group III mGlu receptors function. This is in line with *in vitro* electrophysiological recording of SNr neurons showing that DA depletion with reserpine or haloperidol treatment drastically reduces the inhibitory effects of the group III mGlu receptor agonists L-AP4 on GABAergic but not on glutamatergic transmission (Wittmann et al., 2002). The net effect of group III agonists in the SNr may thus be totally different depending on the level of dopaminergic activity. In control condition, ACPT-I may prominently reduce striatonigral GABAergic transmission, whereas it may preferentially reduce subthalamonigral glutamatergic activity in DA depleted condition, ultimately increasing or decreasing SNr activity, in line with our behavioral and cellular effects. In this connection, involvement of different pathways depending on dopamine tone has also been reported to sustain differential behavioral effect of intrastriatal injection of L-AP4 in control and unilaterally 6-OHDA lesioned animals (Zhang and Albin, 2000).

It is noteworthy that ACPT-I treatment produces restricted regional effects in the SNr. In the 6-OHDA lesioned animals, ACPT-I induces a normalization of CoI mRNA expression in the lateral SNr, without significantly affecting the central and medial parts of the structure. The effects of ACPT-I in controls also appear to be selective to the lateral part of the structure. To our knowledge, the regional specific expression of group III mGlu receptors in this structure has never been assessed, and our

results suggest a prominent function and/or expression onto the synapses that contact the most lateral part of the SNr. Moreover, this regionalized effects support the idea that the systemic treatment with group III agonists mainly affects the motor part of the BG output structure.

Altogether, these results provide the first experimental evidence that systemic administration of a group III mGlu receptor orthosteric agonist may represent an alternative pharmacological strategy to the L-DOPA treatment in an early symptomatic stage of PD. Interestingly, our cellular results support the idea that, when administered via a systemic route, these agonists may not act prominently at the GP level to produce their motor beneficial effects, but preferentially at the STN level. Moreover, the motor deficits induced by ACPT-I in sham animals, in opposition with the palliative effects observed in lesioned rats, further emphasize the strong functional interaction between the dopaminergic system and the group III mGlu receptors.

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