The Dopamine Agonist Piribedil with L-DOPA Improves Attentional Dysfunction: Relevance for Parkinson’s Disease

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ABSTRACT

Cognitive deficits are often associated with motor symptoms in Parkinson’s disease. This study investigates the ability of piribedil [(methylenedioxy-3,4 benzyl)-4 pyperazinyl-1]-2 pyrimidine, a D2/D3 dopamine (DA) receptor agonist with antagonist activity at α2A-adrenoceptors, to restore motor and attentional deficits in nigrostriatal 6-hydroxydopamine-lesioned rats. Subjects were trained to depress a lever, detect a stimulus occurring after variable foreperiods, and release the lever quickly afterward. Striatal DA depletions produce deficits in the timing of foreperiods and prolong reaction times. Although a subchronic treatment with piribedil (0.1–2 mg/kg) is not effective, a dose of 0.3 mg/kg administered for 3 weeks significantly reverses the akinesia deficits produced by the striatal dopamine depletion and progressively improves attentional deficits. When coadministered with the dopamine prodrug L-3,4-dihydroxyphenylalanine (L-DOPA) (3 mg/kg), piribedil (0.3 mg/kg) promotes a rapid and full recovery of preoperative performance. These results suggest that administration of L-DOPA in combination with piribedil in a chronic treatment as either initial or supplemental therapy for Parkinson’s disease might improve cognitive functions while reducing the risk for motor complications.

Since the discovery that patients with Parkinson’s disease (PD) suffer from a dopamine deficiency in the basal ganglia, the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) has been successfully administered to supplement the depleted DA stores. The development of motor fluctuations and abnormal involuntary movements (dyskinesia) is, however, a common side effect of long-term treatment with L-DOPA. DA receptor direct agonists are now commonly used as initial therapy to limit the exposure to L-DOPA and delay the onset of dyskinesia (Jenner, 1995).

Among the current DA agonists acting directly on synaptic receptors in the striatum, piribedil [(methylenedioxy-3,4 benzyl)-4 pyperazinyl-1]-2 pyrimidine) is a centrally acting drug, that shows selectivity for both D2 and D3 dopaminergic receptors and significant antagonistic action on α2A-adrenergic receptors (Millan et al., 2001). In the rat brain piribedil revealed comparable affinities for the dopamine D2- and D3-like receptors and very low affinity for the D1-like receptors (Millan et al., 2002). The anti-akinetic activity of piribedil was first reported in reserpinized rats and in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primate (Smith et al., 1996, 2002). Its efficacy in controlling motor symptoms of PD patients with an incidence of dyskinesia lower than that occurring in patients treated with L-DOPA was recently shown (Montastrauc et al., 1999; Tan et al., 2003). Chronic treatment with piribedil for a 6-month period in combination with L-DOPA is well tolerated and significantly improves motor symptoms in patients with non-fluctuating PD (Ziegler et al., 2003). In addition to the classic motor symptoms, several cognitive deficits are observed in non-demented patients with PD, even at the early stages of the disease (Dubois and Pillon, 1997). The pattern of cognitive impairment includes deficits of executive functions, such as planning and working memory (Brown and Marsden, 1990; Owen, 2004) and attentional deficits resembling those produced by frontal lobe damage (Lewis et al., 2003). In the MPTP-treated marmosets, it has been reported that, in par-
allel to its efficacy in reducing behavioral motor deficits, piribedil induced increased vigilance and awareness compared with L-DOPA (Smith et al., 2002). Piribedil, probably through its α2A-adrenergic antagonist properties, was shown to increase acetylcholine release both in frontal cortex and in the hippocampus (Smith et al., 2002; Gobert et al., 2003). This action may be related to its facilitatory influence upon cognitive function.

Beside motor improvement, the possibility of restoring cognitive functions (memory, reaction time, speed of information processing, etc.) with DA agonists in PD represents a new challenge. Thus, the aim of the present study was to test the effects of piribedil on motor and cognitive deficits in a rat model of early PD. The nigrostriatal dopamine pathway was damaged bilaterally by an intrastratial infusion of the neurotoxin 6-hydroxydopamine (6-OHDA), which induces a progressive and selective loss of DA neurons. It has long been shown that DA depletion in the striatum produces profound deficits in the reaction time (RT) paradigm in rats (Spirduso et al., 1985; Amalric and Koob, 1987; Brown and Robbins, 1991; Amalric et al., 1995; Smith et al., 2000; Courtiere et al., 2005). We have previously found that rats trained to react quickly to a visual cue occurring after a variable interval are slower to react to the cue and sometimes impaired in the timing of the various intervals, depending on the extent of striatal DA denervation (Amalric et al., 1995). These deficits may be related to some extent to the akinesia, assessed by increased RTs (Berry et al., 1999), and attentional dysfunction that are commonly seen in PD patients (Gauntlett-Gilbert and Brown, 1998). In the present study, we have tested the efficacy of piribedil and L-DOPA alone or in conjunction to restore motor and cognitive deficits in 6-OHDA-lesioned rats trained in the RT task.

Materials and Methods

Experiment 1: Locomotor Activity

**Animals.** Male Wistar rats weighing 280 to 300 g on arrival from Charles River (Lyon, France) were housed in groups of two per cage with food and water available ad libitum and maintained in temperature-controlled conditions with an alternating 12 h light/dark cycle (lights on at 7:00 AM). Behavioral measurements were conducted during the animals’ light cycle. All procedures were in strict accordance with the French Ministère de l’Agriculture et de la Pêche, Décret No. 87-848, October 19, 1987, and with the European Communities Council directive of November 24, 1986 (86/609/EEC).

**Apparatus.** A bank of 16 individual wire (top, floor, and front door) and Plexiglas (side walls) photocell cages was used to measure the locomotor activity. Each cage (40 × 25 × 23 cm) was fitted with two parallel horizontal infrared beams, 1 cm above the floor, located across the long axis of the cage (Imetronic, Pessac, France). Beam interruptions were accumulated over 1-min intervals and recorded in bins of 1 min by a one-line input to a microcomputer. The animals were familiarized with the experimental cages during a 3-h session, one day before the test session. On the day of testing, the spontaneous locomotor activity was monitored for 90 min before drug treatment. Four groups of animals (n = 3 per group) received different doses of piribedil (0, 1, 3, or 10 mg/kg) in a different order of injection following a pseudo-random Latin-square design. Injections were performed every 3 days, and the animals were placed immediately afterward in the locomotor activity cages for a total duration of 180 min. After a washout period of 10 days, selected animals received either 0.9% NaCl (n = 4) or piribedil 1.0 mg/kg (n = 8) once a day for 5 consecutive days, and the locomotor activity was recorded for 180 min.

**Statistical Analysis.** Data analysis was carried out using a two-factor analysis of variance (ANOVA). When animals were injected according to a Latin square design, we first analyzed the effects of the order of injections among the different groups. If no significant effect was found, differences between various doses over time were subjected to a two-factor ANOVA, with the different groups (doses of piribedil) as the independent factor and time as the repeated measure. When the ANOVA revealed a significant effect, post hoc comparisons were carried out using the Newman-Keuls test. The significance level was taken to be p < 0.05.

Experiment 2: Reaction Time Task

**Animals.** Male Wistar rats (Charles River), weighing 115 to 120 g at the start of the experiment, were housed in groups of two and maintained on a 12-h light/dark cycle with lights on at 7:00 AM. In the reaction time task, rats were initially food-deprived for 24 h at the start of training and subsequently food-restricted to 15 to 20 g of laboratory chow per rat per day for the duration of the experiment to maintain them at 80 to 85% of the free food body weight. Water was available ad libitum.

**Apparatus and Operant Procedure.** Experiments were conducted in standard experimental chambers (23 × 22 × 30 cm; Campden Instruments, Cambridge, UK) placed in sound attenuated cubicles. The boxes were controlled, and the data were collected on line by a PC computer and laboratory interface (Paul Fray, Inc., Cambridge, UK). A retractable lever, located 5 cm above the grid floor and 4 cm below the cue light (2.8-W bulb), was extended immediately after the animals were placed in the experimental chambers. After completion of a successful trial, the food pellet was delivered to the food magazine in <1 s. The temporal resolution of the instrumental setup was 10 ms. Animals were initially trained to lever press for a 45-mg food pellet (P. J. Noyes Company, Inc., Lancaster, NH) on a schedule that provided one food pellet for every lever press (fixed ratio 1 reinforcement schedule). A force of 0.8 N on the lever was required to operate the switch closure. After the rats successfully responded for >100 pellets, they were trained to hold down the lever until the onset of a cue-light located above the lever. To receive a food pellet during this phase of training, rats had to wait for the cue-light and then release the lever with no time limit. The interval between lever press and the cue-light onset was then progressively increased by steps of 50 ms for up to 1250 ms, after five consecutive correct trials. At the final step, the intervals were fixed and randomized between four foreperiods (500, 750, 1000, and 1250 ms) to maintain a high level of attention to the cue onset and prepare the motor response. RT was measured in milliseconds between the presentation of the cue-light and the release of the lever. The RT restriction was then progressively decreased from 2000 to 600 ms over 40 to 50 additional sessions. Each session ended after 100 trials, which animals typically achieved within 10 to 15 min during the prelesion testing period. Correct responses were those in which the rats released the lever with RTs below the 600-ms time limit. Incorrect responses were not rewarded and divided into two categories. If the lever was released before the presentation of the cue-light, it was recorded as an incorrect response and termed a premature response. Likewise, if the lever was released with RTs above the 600-ms restriction time, it was recorded as an incorrect response and was termed a delayed response. Mean RTs averaged 300 ms at the final part of the test.

**Bilateral Striatal Dopamine Lesions.** Animals were anesthetized by an i.m. injection of xylazine (15 mg/kg) and ketamine (100 mg/kg) and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) with the incisor bar positioned by steps of 50 ms for up to 1250 ms, after five consecutive correct trials. At the final step, the intervals were fixed and randomized between four foreperiods (500, 750, 1000, and 1250 ms) to maintain a high level of attention to the cue onset and prepare the motor response. RT was measured in milliseconds between the presentation of the cue-light and the release of the lever. The RT restriction was then progressively decreased from 2000 to 600 ms over 40 to 50 additional sessions. Each session ended after 100 trials, which animals typically achieved within 10 to 15 min during the prelesion testing period. Correct responses were those in which the rats released the lever with RTs below the 600-ms time limit. Incorrect responses were not rewarded and divided into two categories. If the lever was released before the presentation of the cue-light, it was recorded as an incorrect response and termed a premature response. Likewise, if the lever was released with RTs above the 600-ms restriction time, it was recorded as an incorrect response and was termed a delayed response. Mean RTs averaged 300 ms at the final part of the test.

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striatum at the following coordinates: anteroposterior +0.2 mm; lateral ±3.5 mm, and dorsoventral −4.8 mm (from skull) according to the bregma. The sham control group received the vehicle alone (ascorbate solution, 0.1 mg/ml) in the dorsal striatum. The infusion was made with a micropump over 9 min using a 10-μl Hamilton microsyringe, connected by a Tygon tubing fitting to the 30-gauge stainless steel injector needles. After surgery rats were allowed a 7-day recovery period.

**Drugs.** Piribedil (Trivastal; Servier, Paris, France) was dissolved in 0.9% saline and injected i.p. in a volume of 1 ml/kg. L-DOPA methylster (Sigma-Aldrich) was dissolved with a DOPA decarboxylase (benserasez, 15 mg/kg) (Sigma-Aldrich) in a 0.9% saline solution and injected i.p. in a volume of 1 ml/kg.

**Experimental Procedures.** After training lasting on average 2 to 3 months, rats underwent the surgical procedure (6-OHDA lesions). Behavioral testing resumed on postoperative day 8 and the 6-day per week testing schedule continued for 4 additional weeks.

**Dose response of piribedil.** After the recovery period, the effects of 6-OHDA lesions were tested for six sessions (e.g., from days 9 to 14 after surgery) and the subjects (n = 20) were then divided into four subgroups. The different groups were injected with one of four doses of piribedil (0, 0.1, 0.3, or 1.0 mg/kg i.p., n = 5 per dose) and tested 1 h later in the RT task. This procedure allows the drug to reach its maximal effect in the brain. Piribedil was tested in a subchronic treatment of six injections on postoperative days 15 to 20. After a 3-day washout period, the same groups were injected with higher doses of piribedil (2, 6, and 20 mg/kg) for six additional sessions from postoperative days 25 to 30. A sham-operated group of rats (n = 5) received vehicle injections (0.9% NaCl), and rats were tested in the same conditions from days 9 to 30 postsurgery.

**Chronic treatment with piribedil or L-DOPA.** After a 7-day recovery period, the 6-OHDA lesion or surgery effects were tested over six sessions. The animals (sham n = 32, 6-OHDA n = 60) were then divided into four subgroups, receiving one of the different treatments every day for 3 weeks (from days 16 to 40 postsurgery): sham groups injected with 0.9% NaCl (n = 12), 0.3 mg/kg piribedil (n = 6), 3.0 mg/kg L-DOPA (n = 7), and a combination of 0.3 mg/kg piribedil + 3.0 mg/kg L-DOPA (n = 7); lesion groups injected with 0.9% NaCl (n = 22), 0.3 mg/kg piribedil (n = 18), 3.0 mg/kg L-DOPA (n = 7), and a combination of 0.3 mg/kg piribedil + 3.0 mg/kg L-DOPA (n = 13). The dose of 0.3 mg/kg piribedil was selected on the basis of the results obtained in the first experiment. The dose of 3.0 mg/kg L-DOPA was selected on the basis of previous studies showing that this dose induces no dykinesic effects over a 3 weeks of treatment (Henry et al., 1999) and reduces 6-OHDA lesion-induced deficits in the same task (Turle-Lorenzo et al., 2005). The subjects received the combination of 3.0 mg/kg piribedil + 3.0 mg/kg L-DOPA (n = 20) and 0.3 mg/kg piribedil and 1 h later were tested in the RT task.

**Histology.** At the end of the experiment, animals were killed by decapitation. The brains were then removed and frozen to −80°C. Coronal 10-μm tissue sections were cut at −20°C using a microtome cryostat (Leica CM3050) at the level of the striatum.

The binding of [3H]mazindol to dopamine uptake sites in the striatum was measured according to the procedure described by Javitch et al. (1985). In brief, sections were air-dried and rinsed for 5 min at 4°C 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 (specific activity 17 Ci/mmol; PerkinElmer Life and Analytical Sciences, Zaventem, Belgium) in 50 mM Tris buffer containing 300 mM NaCl and 5 mM KCl added with 0.3 mM desipramine to block the noradrenergic transporter. Nonspecific binding was determined by incubating some sections in the same solution plus 30 μM benztpine. Sections were rinsed twice for 3 min in the incubation medium without mazindol and for 10 s in distilled water and were air-dried. Autoradiographs were generated by apposing the sections to a [3H]-sensitive screen (Raytest, Courbevoie, France) for 7 days and were further quantified with a beta imager (Fuji Bas 5000).

The surface of bilateral striatal 6-OHDA lesions was estimated by delimitating the extent of the lesioned areas in each hemisphere. The neuronal loss was measured in the lesioned areas by quantifying gray layers, which were converted to optical density using external standards (calibrated density step tablet; Kodak). As no difference was found in either the surface or the optical density measured on each side of the brain, the values obtained were averaged for statistical analysis. The mean optical density of the lesioned areas was then compared with the control optical density measured on the same striatal region in sham-operated animals and expressed as a percentage of control values.

**Data and Statistical Analysis.** The number of correct, premature, and delayed responses were collapsed across each session by a block of 6 days (e.g., weeks). The data were analyzed over time (before and after 6-OHDA lesions and during drug treatments: vehicle, piribedil, L-DOPA, or coadministration of piribedil and L-DOPA) using an overall two-way analysis of variance (ANOVA), with one between-subject factor (GROUP) and one within-subject factor (WEEK). As previously found, depending on the extent of striatal DA denervation produced by 6-OHDA infusions, the increase of RTs and delayed responses after the cue may be associated or not with an increase of premature responses before the cue onset, reflecting impulsivity and attentional dysfunction in the timing of the various intervals preceding the cue (Amalric et al., 1995). Chronic piribedil or L-DOPA treatment was thus separately analyzed in the group exhibiting delayed responding to the cue with no premature responding (named kinetic group) and in the group expressing both deficits (named attentional group). One-way ANOVAs followed by post hoc tests (Fisher’s PLSD test) were used for multiple pairwise comparisons within each group when appropriate. All statistical analyses procedure were performed using Statview 5.0 program (Abacus concept). RTs >100 ms (corresponding to real detection of the cue in contrast to coincident lever release) and <800 ms were analyzed in selected sessions of the pre- and postoperative period (before and after drug treatment) to further examine the individual distribution of RTs. The distribution of RTs was plotted as a percentage of the total number by 50-ms bins (frequency) ranging from 100 to 800 ms and averaged for all subjects of a group. To analyze the delay-dependent speeding of the RT, RTs were averaged as a function of the various foreperiods in all subjects during a pre- and postoperative session before and after pharmacological treatment and were submitted to a one-way ANOVA with two withinsubject factors (pre/postlesion and the four foreperiods). In addition, the distribution of premature responses (expressed as a percentage of the total number of premature responses by 50- or 250-ms bin) was further analyzed with regard to the various foreperiods in representative pre- and postoperative sessions (before and after chronic treatment). Data were analyzed using a two-way ANOVA with two within-subject factors: pre/postlesion sessions and foreperiods.

**Results**

**Experiment 1: Locomotor Response to Piribedil**

As shown in Fig. 1, administration of piribedil transiently decreased rats’ locomotor activity within the first 30 min with no further changes over the 180-min testing period. After a nonsignificant effect of the order of injections of piribedil at different doses (P = 0.4), the ANOVA testing the effects of piribedil over time demonstrated a significant main effect of time (F17, 51 = 31.02, p < 0.01) and a significant dose × time interaction (F51, 745 = 4.43, p < 0.01). Subsequent analysis performed during the exploratory phase of the test (e.g., the first 30 min after piribedil injection) (Fig. 1, inset) showed a clear depressant short-term effect whatever the dose tested. Therefore in the following experiments, the effects of piribedil on RT performance were tested 1 h after systemic injection. Data showed that during the period of the
testing, there was no tolerance to the repeated injection of piribedil because subchronic treatment with a dose of 1.0 mg/kg for 5 consecutive days did not modify the locomotor response, which was depressed in the same proportion on the 5th day as on the 1st day (data not shown).

**Experiment 2: Reaction Time Performance**

**Subchronic Treatment with Piribedil.** At completion of the training phase, all animals reached a preoperative level of 65 to 70 correct responses with incorrect responses distributed among 25 to 30 premature and 5 delayed trials per 100-trial session. There was no significant difference in the prelesion baseline values for any parameter of motor performance among the groups. As illustrated in Fig. 2, striatal 6-OHDA lesions impaired RT performance as indicated by a significant decrease in the number of correct responses compared with preoperative levels (main lesion effect $F_{1,16} = 22.51$, $p < 0.01$; Fisher’s PLSD test). Comparisons between groups showed no significant difference in correct, premature, or delayed responses before and after 6-OHDA lesions. Therefore, responses were pooled. The decrease in correct performance was dependent upon variations of premature and delayed responses as revealed by a significant overall effect of lesions on these parameters (main lesion effect $F_{1,16} = 5.6$ and 18.67 for premature and delayed responses, respectively; all $p < 0.05$). Piribedil treatment significantly modified the number of correct, premature, and delayed responses over time ($F_{3,48} = 24.09$, 5.24, and 9.6, respectively; all $p < 0.01$) with no significant differences among subgroups treated with different doses. At low doses, 0.1 to 1 mg/kg piribedil had no significant effect on the deficits produced by the 6-OHDA lesions, as all of the animals exhibited the same level of premature and delayed responses as observed during the first postoperative week (Fig. 2). At the higher doses of 6 and 20 mg/kg, piribedil markedly impaired RT performance by enhancing the 6-OHDA-induced increase in the number of delayed responses ($p < 0.05$; Fisher’s PLSD test). Piribedil at 20 mg/kg decreased the number of correct responses to 24 trials/session as early as the 2nd day of injection. This effect lasted throughout the 6 days of treatment (not shown). In addition, a tendency to increase premature responses was also observed at that dose. Because piribedil at doses $>2$ mg/kg produced deleterious effects on RT performance and lower doses were not active when tested in a subchronic 6-day treatment, the duration of treatment was then increased for up to 3 weeks of daily injection with a low dose of piribedil (0.3 mg/kg).

**Chronic Treatment with Piribedil or L-DOPA.** As found in a previous experiment (Amalric et al., 1995), 6-OHDA lesions disrupted performance by increasing both delayed and premature responding in 63% of subjects ($n = 38/60$), whereas a selective effect on delayed responses was observed in the remaining 37%. The effects of piribedil or L-DOPA were then separately analyzed in these two groups (e.g., attentional versus akinetic, see Materials and Methods).

**Effects of Piribedil or L-DOPA as a Single Treatment.** The striatal DA depletion severely impaired RT performance by dramatically increasing the number of premature responses (Fig. 3A). No recovery of preoperative levels was observed for the entire 4-week testing period (baseline versus postlesion, 1st, 2nd, and 3rd weeks for correct responses, $p < 0.05$; Fisher’s PLSD test after significant ANOVA). In addition, the number of delayed responses progressively increased over time and remained significantly higher than before lesions ($p < 0.05$ baseline versus weeks 1 and 3). As
shown in Fig. 3B, 0.3 mg/kg piribedil gradually reduced the number of premature responses over time with no recovery of the preoperative level of performance, however (prelesion versus postlesion only, $p < 0.05$; Fisher’s PLSD test after significant ANOVA). In contrast, piribedil increased the number of delayed responses at the 2nd week of treatment ($p < 0.05$; Fisher’s PLSD test). A similar pattern of responses was observed after 3.0 mg/kg L-DOPA chronic treatment, although the decrease of premature responses at the last week of treatment (albeit nonsignificant) led to an improvement of the correct performance over time (3rd week versus postlesion only, $p < 0.05$; Fisher’s PLSD test after significant ANOVA). L-DOPA had no effect on the number of delayed responses at any time post-lesion.

**Effects of Piribedil and L-DOPA Coadministration.**

As illustrated in Fig. 4, there was a significant improvement of RT performance in rats treated with a combination of the two drugs at same doses. The number of correct responses gradually returned to preoperative levels over time (postlesion versus 3rd week, $p < 0.01$; prelesion versus 1st and 2nd weeks only, $p < 0.05$). The increased number of premature responses was significantly reduced at the 1st week of cotreatment and remained significantly lower than the postlesion level for the total duration of the testing period (post hoc test after significant one-way ANOVA, postlesion versus 1st, 2nd, and 3rd weeks, $p < 0.05$). Piribedil and L-DOPA coadministration had a biphasic effect on the number of delayed responses, enhancing the deficit produced by 6-OHDA lesions short-term while reversing it at the last week of treatment (2nd week significantly different from prelesion and 3rd week, $p < 0.01$) (Fig. 4). Piribedil or L-DOPA injected alone or in combination had no effect on the performance of sham-operated animals (Table 1).

**Effects of Piribedil and L-DOPA Coadministration on Attentional Deficits.**

To further investigate the nature of the premature responses produced by the 6-OHDA lesion in relation to the variable foreperiods, we analyzed the distribution of these responses (plotted by 50- or 250-ms bins) in pre- and postoperative sessions, at day 13 postlesion, in comparison with the last day of piribedil and L-DOPA cotreatment (Fig. 5, A and B). According to classic models of motor preparation using a simple RT procedure, when variable foreperiods are equiprobable and randomized within a series of trials, the conditional probability of the cue onset increases as time elapses, leading to an increased level of motor preparation, which results in faster RTs as a function of foreperiod duration (Brown and Robbins, 1991). Furthermore, the distribution of the premature responses was not linear after the second foreperiod (750 ms) but rather presented peaks of responses after the value of the cue onset (Fig. 5A). This
suggestions that the animals were able to use the knowledge of the variable foreperiods to prepare their response. In addition, logically the proportion of premature responses increased as a function of time (significant main effect of foreperiods: $F_{3,27} = 14.71, p < 0.05$) (Fig. 5B). 6-OHDA lesions disrupted this pattern, and the proportion of premature responses was found to be similar in the different foreperiods (significant interaction surgery $\times$ foreperiods: $F_{3,27} = 3.39$, $p < 0.05$). Interestingly, the lesions did not affect the knowledge of the duration of the foreperiod values since the peaks were preserved. As illustrated in Fig. 5B, the pattern of premature responses increasing as a function of foreperiod length in a preoperative session ($F_{1,36} = 8.64, p < 0.05$, significant difference in foreperiods 3 and 4 compared with foreperiods 1 and 2, $p < 0.05$; Fisher’s PLSD test) was disrupted by the lesion ($F_{3,36} = 1.2$, N.S.) (Fig. 4B). After chronic treatment with piribedil and L-DOPA, the pattern of preoperative premature responses was fully recovered (significant difference in foreperiods 3 and 4 compared with foreperiods 1 and 2, $p < 0.05$; Fisher’s PLSD test after significant treatment $\times$ foreperiod interaction: $F_{3,36} = 12.1, p < 0.01$).

**Effects of Piribedil and L-DOPA Coadministration on Motor Preparation.** The effects of 6-OHDA lesions were further investigated on the motor preparatory mechanisms by examining the variation of RTs with regard to the different foreperiods (Fig. 6). Preoperatively, RTs were found to be faster as the preparatory level increases (overall main effect of foreperiod: $F_{2,27} = 22.21, p < 0.01$). RTs were found to be significantly shorter after foreperiods 3 and 4 compared with the first foreperiod ($p < 0.05$; Fisher’s PSLD test, after significant ANOVA: $F_{3,36} = 4.41, p < 0.01$), suggesting that the animals have used the conditional probability of cue occurrence to prepare their response. 6-OHDA lesions disrupted this pattern (nonsignificant treatment $\times$ foreperiod interaction: $F_{6,54} = 0.9$) (Fig. 6). In contrast, a significant delay-dependent speeding of RTs was found after cotreatment with piribedil and L-DOPA in the last session of testing (day 36 postlesion $F_{3,36} = 6.8, p < 0.01$). The mean RTs in the two longest foreperiods were found to be significantly faster than in the shorter foreperiods ($p < 0.05$; Fisher’s PLSD test).

**Effects of Piribedil on Akinesia.** As illustrated in Fig. 7A, 6-OHDA-lesioned animals injected with vehicle exhibited a significant decrease in the number of correct responses in the first postoperative week compared with preoperative levels (mean correct 64.3 $\pm$ 2.9) and remained at a level of 57 $\pm$ 3.3 correct responses throughout the 4-week testing period ($F_{8,32} = 5.7, p < 0.01$). The decreased number of correct responses was mainly due to a significant increase in the number of delayed responses ($F_{8,32} = 6.69, p < 0.01$) with no change in premature responding (N.S., not shown). The effects of the lesions were long-lasting, as no recovery was observed for up to 40 days postlesion (preoperative versus all postoperative weeks, $p < 0.05$ for delayed responses; Fisher’s

### Table 1

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<tr>
<td>Piribedil + L-DOPA  ($n = 7$)</td>
<td>Correct</td>
<td>70 $\pm$ 5.57</td>
<td>67 $\pm$ 5.92</td>
<td>75 $\pm$ 6.09</td>
</tr>
<tr>
<td></td>
<td>Premature</td>
<td>23 $\pm$ 4.33</td>
<td>27 $\pm$ 3.76</td>
<td>18 $\pm$ 5.04</td>
</tr>
<tr>
<td></td>
<td>Delayed</td>
<td>5 $\pm$ 1.96</td>
<td>4 $\pm$ 0.85</td>
<td>6 $\pm$ 1.61</td>
</tr>
</tbody>
</table>

Fig. 5. Effects on the pattern of premature responses produced by 6-OHDA lesions and by the coadministration of piribedil (piri) (0.3 mg/kg) and L-DOPA (3 mg/kg). A, distribution of the premature responses (lever release before the cue-light onset) during a preoperative (day – 2; open area), a postoperative (day 13; solid area), and a post-treatment session (day 36; shaded area). The mean percentage of premature responses is plotted as a function of time in a 50-ms bin. B, Mean percentage of premature responses in sham-operated animals.
PLSD test) (Fig. 7A). Piribedil at a low dose of 0.3 mg/kg normalized the number of delayed responses over time (post hoc test after significant one-way ANOVA: postlesion versus 3rd week, \( p < 0.05 \), presurgery versus 1st week only, \( p < 0.01 \); Fisher’s PLSD test). As illustrated in Fig. 7B, the RT distribution showed a shift to the right of the curve toward longer values after lesions and piribedil 0.3 mg/kg induced a shift back toward preoperative values. The increase in mean RTs induced by 6-OHDA lesions (prelesion versus postlesion, \( p < 0.05 \); Fisher’s PLSD test after significant ANOVA) was reduced by a 3-week treatment with piribedil (Fig. 7C).

Histological Results. Examination of \([^{3}H]\)mazindol binding to DA uptake sites was undertaken to assess the extent and the localization of DA terminal loss in the striatum in comparison with sham-operated controls (Fig. 8). Intrastriatal 6-OHDA infusions produced in general a lesion restricted to the dorsal part of the striatum, sparing the fibers located along the lateral ventricle. There was no loss of \([^{3}H]\)mazindol binding in the nucleus accumbens and the olfactory tubercle. Animals found to be inconsistently depleted (e.g., asymmetric lesion or low level of depletion, \( n = 9 \)) were excluded from the statistical analysis. These animals exhibited either no deficits or a transient increase in delayed responses, which rapidly recovered within the 1st week of testing. As classically reported, treatment with 6-OHDA or other neurotoxins [MPTP, rotenone, or methylenedioxymethylamphetamine (Ecstasy)] that damage DA neurons produces variability in the level of DA depletion (Schwarting and Hutton, 1997). In the present study, we therefore performed quantitative analyses in selected animals, belonging to the attentional (\( n = 10 \)) or akinetic (\( n = 11 \)) group, to verify whether the size or intensity of the lesion could explain the behavioral differences. The optical density of \([^{3}H]\)mazindol labeling was quantitatively analyzed and compared at three different anteriority levels (1.2 mm, 0.36 mm, and -0.72 mm) within and outside the core of the lesion. The decrease of \([^{3}H]\)mazindol labeling in the core of the lesion averaged 87% in the attentional group and 58% in the akinetic group in comparison with sham-operated controls (Figs. 8 and 9A). A nonsignificant 15 to 22% decrease in the surrounding striatum was also found in both groups (Fig. 9B). A significant interaction between groups and anteriority levels was found (\( F_{2,38} = 8.79, p < 0.01 \)), showing that the extension of the DA depletion was different rostrocaudally. The main difference was observed in the most rostral levels where a 41% decrease of labeling was found in the akinetic group compared with an 85% decrease in the attentional group (\( p < 0.01 \); simple ANOVA). At the bregma level of anteriority, on sections close to the injection site, the decrease of labeling reached 67 and 89% for the akinetic and attentional groups, respectively, with no significant difference between the two groups. The DA depletion extended caudally to a similar extent in the two groups (65 and 86% decrease, respectively) (Fig. 9A). These results were similar to the measure of the endogenous striatal DA contents assessed by high-performance liquid chromatography in a previous study showing a 74% depletion of tissue levels of DA in the posterior striatum and 53% depletion in the anterior striatum (Amalric et al., 1995). In addition, a significant difference was found between the two groups on the surface of the core of the lesion at these three different levels of anteriority. As illustrated in Fig. 9B, the surface of the lesion core in the attentional group was 1.4 mm\(^2\) rostrally and increased up to 2.2 and 1.8 mm\(^2\) caudally. In contrast, the lesion in the akinetic group (Fig. 8C) was virtually absent rostrally (0.09 mm\(^2\)) and reached a surface of 0.9 and 0.8 mm\(^2\) at caudal levels (\( p < 0.05 \) at the three levels; nonpaired \( t \) test after significant ANOVA: \( F_{1,19} = 18.8, p < 0.01 \)).

Discussion

In the present study, we showed that the \(D_{2}/D_{3}\) dopamine receptor agonist/\(\alpha_{2A}\)-antagonist piribedil is able to counteract the akinetic deficits produced by partial striatal bilateral 6-OHDA lesions in rats trained in a RT task similar to that used to assess motor initiation deficits in PD patients (Gauntlett-Gilbert and Brown, 1998; Muller et al., 2000). A low dose of 0.3 mg/kg piribedil was effective in recovering preoperative performance, provided the animals were chronically injected with the drug. In conditions of extensive 6-OHDA lesions impinging on the rostral regions of the striatum, additional deficits were observed on motor readiness and time estimation that were reduced by piribedil at 0.3 mg/kg and fully reversed with coadministration of L-DOPA at a dose of 3.0 mg/kg.

The positive effects of piribedil in reversing 6-OHDA lesion-induced akinesia and bradykinesia are in line with recent studies conducted in patients with PD showing a signif-
significant improvement of motor symptoms (Montastruc et al., 1999; Ziegler et al., 2003; Simon et al., 2005). Here we found that piribedil is efficient at a low dose (0.3 mg/kg) under a chronic treatment. It is unlikely that these anti-akinetic effects are due to a nonspecific increase of motility, because doses previously found to increase locomotor activity and stereotyped behaviors in rats ranged from 50 to 100 mg/kg (Dourish, 1983). In contrast, lower doses cause depression of activity when tested immediately after injection (Simon et al., 2005). This is consistent with the idea that DA agonists at low doses preferentially activate DA autoreceptors situated on the cell bodies and fibers of neurons in the nigrostriatal system. The inhibition of DA synthesis and turnover may account for the increased number of delayed responses after
piribedil treatment in the attentional group and for the decreased exploratory behavior in the photocell cages.

DA agonist drugs used to control PD vary in their profile of action on D$_1$/D$_2$ and D$_3$ receptors. Piribedil binds preferentially to D$_2$ and D$_3$ receptors and has no significant affinity for the D$_1$ receptors (Millan et al., 2001). Because the blockade of D$_2$ receptors with selective antagonists (raclopride and eticlopride) but not of the D$_1$ or D$_3$ receptor subtypes in rats produced similar RT impairment as those observed here after 6-OHDA nigrostriatal lesions in the same task (Smith et al., 2000), it is tempting to suggest that the reversal of the akinetic deficits is due to piribedil action on striatal D$_2$ receptors. In marmoset monkeys, piribedil improves parkinsonism induced by the neurotoxin MPTP (Smith et al., 1996, 2002), and the improvement of motor function is primarily attributed to activation of postsynaptic receptors in the basal ganglia (Jenner, 1995).

More interestingly is the finding that piribedil treatment may also affect nonmotor deficits in the same task. In addition to the execution of rapid movements, successful completion of the RT task requires that the rats are attentive to the presentation of the cue-light. The loss of control over the “hold period” of the lever when attending to the cue-light after 6-OHDA lesions, illustrated by the increased number of premature responses, therefore suggests a loss of attentional control and a disruption of motor preparation. This leads to impulsive responsiveness and loss of inhibitory control, which is a general feature observed in human and nonhuman primates with frontal lesions (Fuster, 1997; Evenden, 1999). Moreover, there is a considerable overlap between the cognitive deficits observed after damage to the frontal lobes and those described in some patients with PD (Brown and Marsden, 1990; Berry et al., 1999; Lewis et al., 2003; Owen, 2004). Our results indicate that animals with DA denervation spreading to the most rostral regions of the striatum innervated by the prefrontal cortex (e.g., the attentional group) have lost their ability to time the different foreperiods and are therefore unable to use the increasing probability of the cue occurrence to prepare their response to the cue. As a consequence, the delay-dependent speeding of RTs or “motor readiness” seems to be disrupted by the largest DA depletion in the striatum as previously found by others (Brown and Robbins, 1991). Consistent with the idea that a dysfunction of the complex loop between the anterior part of the striatum (e.g., caudate nucleus in human) and the prefrontal cortex underlies the cognitive deficits of PD, excitotoxic lesions in the prelimbic-infralimbic cortical areas in rats have been found to produce similar impairment of motor preparatory processes in the same RT task (Risterucci et al., 2003). The progressive reversal of these deficits by piribedil may be due to its action on D$_3$ and/or $\alpha_2$-adrenergic receptors localized in the frontal cortex. Recent in vitro and in vivo investigations of the properties of piribedil have indeed reported a significant affinity for $\alpha_2$-adrenergic receptors in addition to the DA D$_2$/D$_3$ receptors (Millan et al., 2001). As an $\alpha_2$-adrenergic receptor antagonist, piribedil was found to enhance frontocortical release of acetylcholine (Newman-Tancredi et al., 2002; Gobert et al., 2003). Basal forebrain cholinergic neurons are well known to be involved in attentional processing, and the loss of these neurons from the parkinsonian brain may contribute to attentional dysfunction. In PD patients, cholinergic treatments have had only limited success in treating executive and attentional deficits, whereas some of these are ameliorated with naphthoxazine, a compound with $\alpha_2$-adrenoceptor agonist-like activity (Bedard et al., 1998).

Selective antagonists of $\alpha_2$-adrenergic receptors, such as atipamezole or idazoxan, attenuate motor symptoms (circling behavior) in unilateral 6-OHDA-lesioned rats (Chopin et al., 1999), whereas in MPTP-treated monkeys they facilitate the action of L-DOPA (Bezard et al., 1999) and attenuate dyskinesia (Henry et al., 1999; Grondin et al., 2000). None of these studies, however, have investigated the action of these drugs on cognitive deficits in animal models of PD. We found here that repeated administration of piribedil in conjunction with L-DOPA in rats bearing bilateral 6-OHDA lesions attenuates the attentional dysfunction and reverses the akinetic deficits. Whether the improvement of cognitive deficits involves a selective action on $\alpha_2$-adrenergic receptors, ultimately modulating cholinergic activity, or on DA D$_3$ receptors still remains to be clarified.

Clinical studies recently showed the efficacy of piribedil in the treatment of PD in monotherapy or in conjunction with L-DOPA (Ziegler et al., 2003). When tested in MPTP-treated marmosets, long-term treatment (30 days) with piribedil or L-DOPA produces equivalent reversal of motor deficits over the course of the study. In contrast to L-DOPA, however, piribedil produces a significantly lower degree of dyskinesia (Smith et al., 1996, 2002). This may relate to the recent findings that blocking of $\alpha_2$-adrenergic receptors in PD patients improves L-DOPA-induced dyskinesia without the reappearance of parkinsonian symptoms (Colosimo and Craus, 2003). In the present study, the improvement of 6-OHDA-induced deficits was not associated with abnormal dyskinetic movements at any time postlesion. The initial alteration of the delayed responses in the first weeks of cotreatment, however, is likely to be due to a selective effect of dopamine agonists injected at low dose. This was attributed to a presynaptic action on dopaminergic terminals, therefore primarily reducing DA synthesis. In line with this, L-DOPA treatment was found to produce sedation in de novo parkinsonian patients (Andreu et al., 1999; Muller et al., 2000). In the long-term, chronic treatment with piribedil may produce tolerance to these immediate effects through desensitization of DA receptors and ultimately regulate DA function on postsynaptic receptors.

The effects of piribedil on cognitive functions have not yet been tested in parkinsonian patients, but in elderly patients. Nagaraja and Jayashree (2001) demonstrated, after a randomized, double-blind clinical trial, that piribedil improves global cognitive function in patients with mild cognitive impairment. In young healthy volunteers, piribedil improves alertness and speeds information processing (Schuck et al., 2002). Altogether, these results indicate that piribedil in combination with L-DOPA may have beneficial effects on cognitive function in the early stages of PD.

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References


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