70th birthday Peter Riederer

The effect of piribedil on L-DOPA-induced dyskinesias in a rat model of Parkinson's disease: differential role of α_2 adrenergic mechanisms

Manfred Gerlach¹, Paul Halley², Peter Riederer³, Maarten van den Buuse, PhD²

¹ University of Würzburg, Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Würzburg, Germany

² Behavioural Neuroscience Laboratory, Mental Health Research Institute, Parkville, Australia

³ University of Würzburg, Department of Psychiatry, Psychosomatics and Psychotherapy, and National Parkinson Foundation Centers of Excellence for Neurodegenerative Diseases Research, Würzburg, Germany

Address for correspondence and reprint requests:

Associate Professor Maarten van den Buuse

Mental Health Research Institute

151 Oak Street

Parkville, Melbourne, VIC 3052, Australia

Telephone: +61 3 93892967

Fax: +61 3 93875061

E-mail: m.vandenbuuse@mhri.edu.au

ABSTRACT

Piribedil is a non-ergoline, dopamine D₂/D₃ receptor agonist with α_2 adrenoceptor antagonist properties that has been used in the treatment of Parkinson's disease (PD). Noradrenergic neurotransmission may be involved in the pathogenesis of dyskinesias but its role in the *in vivo* action of piribedil or on different sub-classes of abnormal involuntary movements (AIMs) remains unclear. The aims of this study were therefore (1) to investigate the anti-dyskinetic effects of piribedil on L-DOPA-induced contralateral turning behavior, locomotive dyskinesias (LD), axial dystonia (AD), orolingual dyskinesia (OD) and forelimb dyskinesias (FD) and (2) to compare these effects to the α_2 adrenoceptor antagonist, idazoxan, or the α_2 adrenoceptor agonist, clonidine.

Rats were unilaterally lesioned with 6-hydroxydopamine (6-OHDA) and injected intraperitoneally twice daily with L-DOPA methylester (12.5 mg/kg) and benserazide (3.25 mg/kg). After three weeks, the effects of piribedil (5, 15, 40 mg/kg), clonidine (0.15 mg/kg), idazoxan (10 mg/kg) and combinations of these drugs were scored during two hours.

Pre-treatment with 5 and 40 mg/kg, but not 15 mg/kg of piribedil, reduced turning behaviour and AD, OD and FD but piribedil increased LD at the 15 and 40 mg/kg doses compared to the L-DOPA group. Idazoxan induced similar effects as piribedil (40 mg/kg) except that it had no effect on LD. Idazoxan blocked the effect of piribedil on AD and FD. Clonidine, reduced all AIMs except OD possibly because of its sedative effect. Clonidine blocked the effect of piribedil on AD, OD and FD.

These data suggest a differential involvement of α_2 adrenergic receptors in the action of piribedil on different subclasses of L-DOPA-induced dyskinesias.

Key Words: Parkinson therapy, 6-hydroxydopamine, hemiparkinsonian rat, levodopa, dopamine agonists, α_2 adrenergic receptors

INTRODUCTION

Piribedil, a non-ergoline, dopamine D₂ and D₂ receptor agonist with α_2 adrenoceptor antagonist properties (Creese 1974; Millan, 2010; Millan et al. 2001; Newman-Tancredi et al. 2002), has been used for many years in the treatment of Parkinson's disease (PD) and is effective in controlling all major motor symptoms of PD both as adjunct to L-DOPA (3,4-dihydroxyphenylalanine, levodopa) and as monotherapy (Rascol et al. 2006; Ziegler et al. 2003).

There is pharmacological evidence that noradrenergic neurotransmission is involved in the pathogenesis of L-DOPA-induced dyskinesia that is characterized by debilitating involuntary movements which limit quality of life in patients suffering from PD (Brotchie et al. 2005; Gerlach et al. 2011; Iravani and Jenner 2011; Müller and Russ 2006). For example, the α_2 adrenoceptor antagonist idazoxan has shown anti-dyskinetic efficacy in monkey studies (Fox et al. 2001; Grondin et al. 2000; Henry et al., 1999b). In addition, in clinical trials, idazoxan reduced L-DOPA-induced dyskinesia without altering the antiparkinsonian action of L-DOPA (Rascol, et al. 2001).

In rats, L-DOPA-induced dyskinesia can be modelled via intracerebral injection of the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA) - that damages the nigro-striatal pathway - followed by chronic administration of low doses of L-DOPA (Gerlach and Riederer 1996; Lundblatt et al. 2004). This treatment causes a sensitisation of the contraversive circling response and characteristic abnormal involuntary movements (AIMs) on the body side that is ipsilateral to the lesion with dyskinesia-associated cellular responses (Henry et al. 1999a; Lundblad et al. 2004). These AIMs can be further sub-divided into locomotive dyskinesias (LD), axial dystonia (AD), orolingual dyskinesia (OD) and forelimb dyskinesias (FD). This model has been pharmacologically validated and represents a cost-effective alternative to non-human primates for screening drugs with potential anti-dyskinetic properties (Dekundy et al. 2007; Lundblad et al. 2002). However, it is unclear whether the pharmacology of AIM sub-types is similar.

The aim of this study was to further examine the anti-dyskinetic potential of intraperitoneally (i.p.) administered piribedil in unilaterally 6-OHDA-lesioned rats that received chronic administration of L-DOPA and benserazide, a peripherally active amino acid decarboxylase inhibitor. The study focused on the potential differences in

the effect of piribedil on the various sub-classes of AIMS. Furthermore, to elucidate the role of α_2 adrenergic receptors, the effects of piribedil were compared with those of the α_2 adrenergic antagonist, idazoxan, or the α_2 adrenergic agonist, clonidine (Grondin et al. 2000; Henry et al. 1999b; Watling 2006). Some of the results have been presented in preliminary form elsewhere (Halley et al. 2010).

MATERIALS AND METHODS

Animals

Animal experiments were carried out at the Behavioural Neuroscience Laboratory, Mental Health Research Institute, Melbourne, Australia. All procedures complied with the NIH Guide for the care and use of laboratory animals. The University of Melbourne Animal Experimentation Ethics Committee approved all surgical techniques, treatments and experimental protocols.

Sprague-Dawley rats (Animal Resource Centre, Canning Vale, Western Australia) weighing 250-300 g were housed in pairs on a 12-h light/dark cycle with an ambient temperature of $21 \pm 2^\circ\text{C}$. Rats had access to standard rat chow and water *ad libitum*.

Preparations and administration of drugs

All drugs were of the highest purity and were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Piribedil was supplied by Desitin Arzneimittel GmbH, Hamburg, Germany.

Piribedil was administered i.p. at 5, 15 and 40 mg/kg based on previous findings in rats showing behavioural effects in this dose range (Corrodi et al. 1972; Newman-Tancredi et al. 2002; Turle-Lorenzo et al. 2006). To elucidate the role of the α_2 adrenergic receptor, the effects of piribedil were compared with 0.15 mg/kg clonidine and 10 mg/kg idazoxan (Grondin et al. 2000; Henry et al. 1999b; Watling, 2006). Given the sedative and hypothermic properties of clonidine, its dose was much lower than equimolar (25.7 mg/kg) to 40 mg/kg piribedil while still reported to elicit behavioural responses in rodents (Bardgett et al. 2008; Herradon et al. 2008). Similarly, the dose of idazoxan was lower than the equimolar dose (24) to 40 mg/kg piribedil as this was the highest dose reported in the literature (e.g. Nakagawa et al. 2004).

L-DOPA was administered as the methyl ester form, as it is more stable and soluble than non-esterified DOPA. L-DOPA methyl ester is rapidly de-esterified *in vivo* by

non-specific esterases to form L-DOPA (Cooper et al. 1984). In all experiments L-DOPA was administered at the same time as benserazide.

All drugs were dissolved in sterile saline (except where indicated) and injected at a volume of 1 ml/kg of body weight. Because piribedil was found to be insoluble in both water and DMSO, it was suspended using a standard suspension vehicle (0.9% w/v NaCl, 0.5% w/v Na-carboxy-methylcellulose and 0.005% v/v benzyl alcohol and 0.004% v/v Tween 80).

6-OHDA lesion

The animals were treated i.p. with pargyline (5 mg/kg) and desipramine (20 mg/kg) to enhance the effect of 6-OHDA on dopamine neurons and to block its effect on noradrenergic neurons, respectively. The rats were then anesthetized with isoflurane and mounted in a Kopf stereotaxic frame equipped with a mouth mask to deliver gas anesthetic. Unilateral 6-OHDA lesion of the median forebrain bundle was done in the right side of the brain. Stereotaxic coordinates were -2.8 mm from bregma, 2 mm lateral and 9 mm ventral to the skull surface (Paxinos and Watson 1986). A volume of 2.5 µl of 6-OHDA HCl solution (5 mg/ml with 0.1% ascorbic acid) was injected over 5 minutes. The rats were allowed three weeks of post-operative recovery.

Experimental design

Three weeks following 6-OHDA-lesion, rats were injected i.p. twice per day (around 9 a.m. and around 5 p.m.) with 12.5 mg/kg of L-DOPA methyl ester and 3.25 mg/kg of benserazide. This was done in three separate cohorts of rats, the results of which were pooled. Because we reported on the development of L-DOPA-induced turning behaviour in detail before (Gerlach et al. 2004), we only verified turning responses on day 7 and day 14 after the start of chronic injections. The results from these two measurements were used to select the best responders to L-DOPA/benserazide while non-responding animals were euthanized. The selected animals (n=29) were subsequently tested four times for turning behaviour and AIMs, with 3-4 days between testing while continuing to receive daily treatment with L-DOPA/benserazide. Treatments on the testing days were determined pseudo-randomly, with none of the animals receiving the same treatment or treatment combination twice. The first two of these testing days focused on the effects of piribedil, clonidine or idazoxan, while on the last two testing days the animals received combinations of 40 mg/kg of piribedil with clonidine or idazoxan. Control responses were obtained after treatment with

vehicle and L-DOPA/benserazide; piribedil responses were obtained after treatment with either 5, 10 or 40 mg/kg piribedil and L-DOPA/benserazide; adrenergic drug responses were obtained after treatment with 0.15 mg/kg clonidine or 10 mg/kg idazoxan and L-DOPA/benserazide. These drugs were administered 5 min before administration of L-DOPA/benserazide.

Assessment of dyskinesia

At days of measuring the rotational behaviour, rats were removed from their home cages, injected with either a drug or vehicle, and placed immediately in an automated Rotacount system (Columbus Instruments, Columbus, OH, USA), allowing unbiased recording of full and partial clockwise and counter-clockwise turns. The number of full clockwise (ipsiversive) and counter-clockwise (contraversive) turns was recorded every five minutes during two hours.

AIMs were assessed as previously described (Cenci et al. 1998) and classified into four subtypes: LD i.e. increased locomotion with contralateral side bias; AD i.e. contralateral twisted posturing of the neck and upper body; OD i.e. stereotyped jaw movements and contralateral tongue protrusion; FD i.e. repetitive rhythmic jerks of dystonic posturing of the contralateral forelimb and/or grabbing movements of the contralateral paw. For each of these four subtypes, a score from 0-4 (0=absence; 4=severe) was given and a total score calculated. AIMs were assessed by an observer every 5 minutes during the two-hour experimental period.

Data analysis

Data obtained from vehicle-treated controls on every testing day were combined to provide a vehicle control group to which the effects of all drug treatments were compared. Data are presented as mean $\pm$ standard error of the mean (SEM). Group data for turning behaviour were compared with analysis of variance (ANOVA) followed by Least-Significant-Difference post-hoc pairwise comparison (Systat 9.0, SPSS Inc., USA). Group data for LD, AD, OD and FD were analyzed with the Kruskal-Wallis test (Systat 9.0) and pair-wise group comparisons were done with Mann-Whitney test (GraphPad Prism 5.01, USA). When $P < 0.05$, differences between groups were considered statistically significant. The Bonferroni correction was used for multiple comparisons where appropriate.

RESULTS

Pre-treatment with 5 mg/kg and 40 mg/kg, but surprisingly not 15 mg/kg of piribedil, led to a reduction of L-DOPA-induced contralateral turning behaviour (Fig 1).

Interestingly, this bell-shaped dose-response profile was also found for the AIMs subtypes AD, OD and FD. In contrast, piribedil treatment appeared to cause a dose-dependent increase in the frequency of LD (Fig. 1).

Pre-treatment of the rats with the selective α_2 adrenergic antagonist idazoxan (10 mg/kg) caused similar effects as seen with the highest dose of piribedil. Thus, the number of contralateral turns and the frequency of AD, OD and LD were significantly reduced (Fig. 1). However, idazoxan treatment had no effect on LD. Idazoxan reversed the effect of piribedil on AD and FD, but it had no effect on LD, OD or the number of contralateral turns (Fig. 1).

Pre-treatment of the rats with the selective α_2 adrenergic agonist clonidine (0.15 mg/kg) reduced the number of contralateral turns and the frequency of LD, AD and FD, but not OD (Fig. 1). Clonidine reversed the effect of piribedil on AD, OD and FD, but not on LD or the number of contralateral turns (Fig. 1).

DISCUSSION

Using the L-DOPA-sensitised unilaterally 6-OHDA-lesioned rat model of dyskinesia we found that pre-treatment with piribedil leads to a reduction of L-DOPA-induced contralateral turnings and most AIMs score in a bell-shaped dose-response profile. This result is most likely caused by the partial (inverse) agonist and antagonist activity of piribedil at dopamine- and α_2 adrenergic receptors, respectively (Creese 1974; Millan et al. 2001; Newman-Tancredi et al. 2002) and can be explained by a differential activation of pre- or post-synaptic receptors at high and low doses. However, our study reveals major differences between sub-classes of dyskinesias including contralateral turning and AIMs, with respect to their *in vivo* pharmacology.

Classically, contraversive circling in rats with unilateral 6-OHDA lesions is used to study the potential of compounds to reverse the motor symptoms of PD (Carey 1986; Gerlach and Riederer 1996; Ungerstedt and Arbuthnott 1970). L-DOPA and selective dopamine D₂ receptor agonists all evoke contraversive rotations in 6-OHDA-lesioned rats as a result of post-synaptic dopamine stimulation and a supersensitivity of dopamine receptors on the lesioned side. Repeated administration of L-DOPA in combination with a peripheral decarboxylase inhibitor sensitises this circling response and also induces AIMs, both of which have been associated with dyskinesia

induction (Lundblad et al. 2002; Lundblad et al. 2004). We found that pre-treatment with the lowest dose piribedil employed (5 mg/kg i.p.) led to a reduction of L-DOPA-induced contralateral turning behaviour and most AIMs scores. These findings indicate that piribedil may alleviate L-DOPA-induced dyskinesias in PD patients. The anti-dyskinetic potential of piribedil in the treatment of PD patients is supported by studies in non-human primates displaying PD-like symptoms after treatment with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Switching from L-DOPA to piribedil (3.0-4.0 mg/kg per orally, once daily) resulted in a sustained decrease in dyskinesia intensity (Smith et al. 2006). In addition, it was shown that repeated administration of piribedil induces less dyskinesia than L-DOPA (Smith et al. 2002). It has been suggested that the reduction in dyskinesias represents additional facets of the pharmacological profile of piribedil such as the α_2 adrenergic antagonistic activity (Smith et al. 2006). Indeed, we found that pre-treatment with the selective α_2 adrenergic antagonist idazoxan induced anti-dyskinetic effects similar to piribedil.

A striking finding of the present results was qualitative differences between sub-classes of AIMs with respect to their pharmacology. The clearest example of this was in LD which, in contrast to all other AIMs sub-categories, was not reduced but increased by piribedil treatment. In addition, idazoxan treatment did not reduce the frequency of LD, unlike all other dyskinesias. This finding suggests that α_2 adrenergic receptors may not be involved in LD. The effect of piribedil on LD may be instead be mediated by its action on dopaminergic receptors but further studies with combinations of piribedil and dopamine receptor antagonists are needed to confirm this possibility.

It has previously been suggested that there are qualitative differences in the neural substrates of different sub-classes of AIMs (Winkler et al. 2002). Specifically, it was suggested that the extent of involvement of proximal limb and axial muscles in AIMs was proportional to the spreading of dopamine depletion from lateral towards medial aspects of the caudate-putamen (Winkler et al. 2002). Our results show that in addition to these regional differences, there are likely consequential pharmacological differences between different AIMs sub-classes.

There were also other examples of differences between dyskinesia sub-classes in the effects of piribedil and idazoxan. It was found that, in contrast to AD, OD and FD,

co-treatment with idazoxan did not reverse the effect of piribedil on the number of contralateral turns, despite reducing this behaviour on its own. This again suggests that in addition to α_2 adrenergic receptors, other mechanisms such as dopamine receptor activation, may be involved in the action of piribedil on the number of contralateral turns. This suggestion was supported by the observation, that after co-treatment with piribedil and clonidine, there was still significant reduction of the number of contralateral turns. Previous studies have similarly suggested that in hemiparkinsonian rats, the number of contralateral turns and the frequency of AIMs are regulated by different pharmacological mechanisms. For example, dopamine D_1 receptor agonists increased the number of contralateral rotations and these effects were enhanced by concurrent 5-HT_{1A} receptor activation but the frequency of D_1 receptor-mediated AIMs were reduced by 5-HT_{1A} receptor activation (Dupre et al. 2007). Unfortunately this previous study did not distinguish between different AIMs categories. Recently, another study showed that, unlike L-DOPA which induces both contralateral turning and AIMs, chronic treatment with dopamine D_2 receptor agonists (bromocriptine, piribedil, ropinirole) induces only contralateral turning without AIMs (Lane and Dunnett 2010).

Surprisingly, similar to idazoxan, pre-treatment with clonidine also displayed apparent anti-dyskinetic effects, as has been shown by others before (Dekundy et al. 2007). However, the results should be interpreted with caution as these low AIMs scores may be due to a sedative effect of clonidine. For example, these animals displayed very little LD or AD because they became too hypoactive after about 10 minutes. However the AIMs that required less effort, such as FD and particularly OD, were still evident, although the former was also significantly less than control. A non-specific mechanism of action of clonidine is also suggested by the observation that it reduced LD, which was insensitive to idazoxan treatment suggesting that it is not mediated by a specific α_2 adrenergic mechanism. The effect of combination treatment with clonidine and piribedil may then be explained by the α_2 adrenergic antagonist properties of piribedil blocking the sedative α_2 adrenergic agonist effects of clonidine. At the same time, the α_2 adrenergic agonist properties of clonidine blocked the effects of piribedil on AIMs scores. Indeed, AIMs scores after this combination treatment were not different with those found after vehicle treatment.

In conclusion, this study showed that pre-treatment with piribedil may lead to a reduction of L-DOPA-induced contralateral turning and AIMs score in a bell-shaped dose-response profile. Comparison of piribedil with the effects of idazoxan and clonidine confirmed a likely involvement of α_2 adrenoceptors in its mechanism of action although detailed analysis of the data revealed that additional pharmacological mechanisms are likely involved as well, e.g. dopamine receptor activation. These findings shed further light on the mechanisms by which piribedil may alleviate L-DOPA-induced dyskinesias in PD patients.

ACKNOWLEDGEMENTS

This study was funded by Desitin Arzneimittel GmbH (Hamburg, Germany) without restriction. The authors are grateful to Dr Wendy Adams for excellent technical assistance and to Dr Olivia Dean for assistance with the behavioural analysis.

REFERENCES

- Bardgett ME, Points M, Ramsey-Faulkner C, Topmiller J, Roflow J, McDaniel T, Lamontagne T, Griffith MS (2008) The effects of clonidine on discrete-trial delayed spatial alternation in two rat models of memory loss. *Neuropsychopharmacology* 33: 1980-1991
- Brotchie JM, Lee J, Venderova K (2005) Levodopa-induced dyskinesia in Parkinson's disease. *J Neural Transm* 112: 359-391
- Carey RJ (1986) Relationship of changes in spontaneous motor activity to spontaneous circling in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. *Exp Neurol* 92: 591-600
- Cenci MA, Lee CS, Bjorklund A (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. *Eur J Neurosci* 10: 2694-2706
- Cooper DR, Marrel C, Testa B, van de Waterbeemd H, Quinn N, Jenner P, Marsden, CD (1984) L-DOPA methyl ester - a candidate for chronic systemic delivery of L-DOPA in Parkinson's disease. *Clin Neuropharmacol* 7: 89-98
- Corrodi H, Farnebo LO, Fuxe K, Hamberger B, Ungerstedt U (1972) ET495 and brain catecholamine mechanisms: evidence for stimulation of dopamine receptors. *Eur J Pharmacol* 20: 195-204

- Creese I (1974) Behavioural evidence of dopamine receptor stimulation by piribedil (ET495) and its metabolite S584. *Eur J Pharmacol* 28: 55-58
- Dekundy A, Lundblad M, Danysz W, Cenci MA (2007) Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behav Brain Res* 179: 76-89
- Dupre KB, Eskow KL, Negron G, Bishop C (2007) The differential effects of 5-HT_{1A} receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat. *Brain Res* 1158: 135-143
- Fox SH, Henry B, Hill MP, Peggs D, Crossman AR, Brotchie JM (2001) Neural mechanisms underlying peak-dose dyskinesia induced by levodopa and apomorphine are distinct: evidence from the effects of the α_2 adrenoceptor antagonist idazoxan. *Mov Disord* 16: 642-650
- Gerlach M, Riederer P (1996) Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man. *J Neural Transm* 103: 987-1041
- Gerlach M, van den Buuse M, Blaha C, Bremen D, Riederer P (2004) Entacapone increases and prolongs the central effects of L-DOPA in the 6-hydroxydopamine-lesioned rat. *Naunyn Schmiedeberg's Arch Pharmacol* 370: 388-394
- Gerlach M, Riederer P, Scheller D (2011) Editorial. Mechanisms underlying and medical management of L-Dopa-associated motor complications. *J Neural Transm* 118: 1659-1660
- Grondin R, Hadj Tahar A, Doan VD, Ladure P, Bedard PJ (2000) Noradrenoceptor antagonism with idazoxan improves L-dopa-induced dyskinesias in MPTP monkeys. *Naunyn Schmiedeberg's Arch Pharmacol* 361: 181-186
- Henry B, Crossman AR, Brotchie JM (1999a) Effect of repeated L-DOPA, bromocriptine, or lisuride administration on preproenkephalin-A and preproenkephalin-B mRNA levels in the striatum of the 6-hydroxydopamine-lesioned rat. *Exp Neurol* 155: 204-220
- Henry B, Fox SH, Peggs D, Crossman AR, Brotchie JM (1999b) The α_2 -adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian

- actions of L-dopa in the MPTP-lesioned primate model of Parkinson's disease. *Mov Disord* 14: 744-753
- Halley P, Riederer P, van den Buuse M, Lohmueller K, Gerlach M (2010) Anti-dyskinetic effects of piribedil on levodopa-induced dyskinesia in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Mov Disord* 25 [Suppl S2]: S209-S209
- Herradon G, Morales L, Gramage E, Alguacil LF (2008) Comparative study of alpha2-adrenoceptors in Fischer 344 and Lewis rats. Evidence for clonidine-induced place aversion. *Life Sci* 82: 1186-1190
- Iravani MM, Jenner P (2011) Mechanisms underlying the onset and expression of levodopa-induced dyskinesia and their pharmacological manipulation. *J Neural Transm* 118: 1661-1690
- Lane EL, Dunnett SB (2010) Pre-treatment with dopamine agonists influence L-dopa mediated rotations without affecting abnormal involuntary movements in the 6-OHDA lesioned rat. *Behav Brain Res* 213: 66-72
- Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA (2002) Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *Eur J Neurosci* 15: 120-132
- Lundblad M, Picconi B, Lindgren H, Cenci MA (2004) A model of L-DOPA-induced dyskinesia in 6-hydroxydopamine lesioned mice: relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* 16: 110-123
- Millan MJ (2010) From the cell to the clinic: A comparative review of the partial D-2/D-3 receptor agonist and alpha(2)-adrenoceptor antagonist, piribedil, in the treatment of Parkinson's disease. *Pharmacol & Therapeutics* 128: 229-273
- Millan MJ, Cussac D, Milligan G, Carr C, Audinot V, Gobert A, Lejeune F, Rivet JM, Brocco M, Duqueyrox D, Nicolas JP, Boutin JA, Newman-Tancredi A (2001) Antiparkinsonian agent piribedil displays antagonist properties at native, rat, and cloned, human α_2 -adrenoceptors: cellular and functional characterization. *J Pharmacol Exp Ther* 297: 876-887
- Müller T, Russ H (2006) Levodopa, motor fluctuations and dyskinesia in Parkinson's disease. *Expert Opin Pharmacother* 7: 1715-1730

- Nakagawa M, Ohgoh M, Nishizawa Y, Ogura H (2004) Dopaminergic agonists and muscarinic antagonists improve lateralization in hemiparkinsonian rats in a novel exploratory Y-maze. *J Pharmacol Exp Ther* 309: 737-744
- Newman-Tancredi A, Cussac D, Audinot V, Nicolas JP, De Ceuninck F, Boutin JA, Millan MJ (2002) Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D₂-like receptor and α_1/α_2 -adrenoceptor. *J Pharmacol Exp Ther* 303: 805-814
- Paxinos G, Watson C (1986) The rat brain in stereotaxic co-ordinates, 2nd edition. Academic press, New York
- Rascol O, Arnulf I, Peyro-Saint Paul H, Brefel-Courbon C, Vidailhet M, Thalamas C, Bonnet AM, Descombes S, Bejjani B, Fabre N, Montastruc JL, Agid Y (2001) Idazoxan, an alpha-2 antagonist, and L-DOPA-induced dyskinesias in patients with Parkinson's disease. *Mov Disord* 16: 708-713
- Rascol O, Dubois B, Caldas AC, Senn S, Del Signore S, Lees A (2006) Early piribedil monotherapy of Parkinson's disease: A planned seven-month report of the REGAIN study. *Mov Disord* 21: 2110-2115
- Smith LA, Tel BC, Jackson MJ, Hansar, MJ, Braceras R, Bonhomme C, Chezaubernard C, Del Signore S, Rose S, Jenner P (2002) Repeated administration of piribedil induces less dyskinesia than L-dopa in MPTP-treated common marmosets: a behavioural and biochemical investigation. *Mov Disord* 17: 887-901
- Smith LA, Jackson MJ, Johnston L, Kuoppamaki M, Rose S, Al-Barghouthy G, Del Signore S, Jenner P (2006) Switching from levodopa to the long-acting dopamine D2/D3 agonist piribedil reduces the expression of dyskinesia while maintaining effective motor activity in MPTP-treated primates. *Clin Neuropharmacol* 29: 112-125
- Turle-Lorenzo N, Maurin B, Puma C, Chezaubernard C, Morain P, Baunez C, Nieoullon A, Amalric M (2006) The dopamine agonist piribedil with L-DOPA improves attentional dysfunction: relevance for Parkinson's disease. *J Pharmacol Exp Ther* 319: 914-923

- Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* 24: 485-493
- Watling K (2006) *The Sigma-RBI Handbook of Receptor Classification and Signal Transduction*. Sigma-RBI Natick
- Winkler C, Kirik D, Bjorklund A, Cenci MA (2002) L-DOPA-induced dyskinesia in the intrastriatal 6-hydroxydopamine model of parkinson's disease: relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* 10: 165-186
- Ziegler M, Castro-Caldas A, Del Signore S, Rascol O (2003) Efficacy of piribedil as early combination to levodopa in patients with stable Parkinson's disease: a 6-month, randomized, placebo-controlled study. *Mov Disord* 18: 418-425

Figure legend

Figure 1: Effects of pre-treatment with piribedil, idazoxan, and clonidine on L-DOPA-induced turning behaviour and subtypes of abnormal involuntary movements (AIMs) in unilateral 6-OHDA-lesioned rats. Piribedil was administered at 5 (P5), 15 (P15) or 40 mg/kg intraperitoneally (P40). Idazoxan was administered at 10 mg/kg (Id) on its own or in combination with 40 mg/kg piribedil (P+Id). Clonidine was administered at 0.15 mg/kg (Cl) on its own or in combination with 40 mg/kg piribedil (P+Cl). * $P < 0.05$ for difference with vehicle-treated rats; $^{\$}P < 0.05$ for difference with piribedil (40 mg/kg) treated rats. Data are mean $\pm$ SEM of $n=20$ rats per treatment.