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Published in:
Experimental Neurology

DOI:
10.1016/j.expneurol.2005.02.002

2005

Pharmacological validation of a mouse model of L-DOPA-induced dyskinesia

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Abbreviations:

DA, dopamine; L-DOPA, L-3,4, dihydroxyphenylalanine; i.p., intraperitoneal; MPTP, 1-methyl-4-phenyl-1,2,5,6,-tetrahydropyridin; 6-OHDA, 6-hydroxydopamine; 5-TH, 5-hydroxytryptamine; NMDA, N-methyl-D-aspartate; PD, Parkinson´s disease

Abstract

Dyskinesia (abnormal involuntary movements) is a common complication of L-DOPA pharmacotherapy in Parkinson´s disease, and is thought to depend on abnormal cell signaling in the basal ganglia. Dopamine (DA) denervated mice can exhibit behavioural and cellular signs of dyskinesia when they are treated with L-DOPA, but the clinical relevance of this animal model remains to be established. In this study, we have examined the pharmacological profile of L-DOPA-induced abnormal involuntary movements (AIMs) in the mouse. C57BL/6 mice sustained unilateral injections of 6-hydroxydopamine (6-OHDA) in the striatum. The animals were treated chronically with daily doses of L-DOPA that were sufficient to ameliorate akinetic features without inducing overt signs of dyskinesia upon their first administration. In parallel, other groups of mice were treated with antiparkinsonian agents that do not induce dyskinesia when administered de novo, i.e. the D2/D3 agonist ropinirole, and the adenosine A2a antagonist KW-6002.

During 3 weeks of treatment, L-DOPA-treated mice developed AIMs affecting the head, trunk and forelimb on the side contralateral to the lesion. These movements were not expressed by animals treated with ropinirole or KW-6002 at doses that improved forelimb akinesia. The severity of L-DOPA-induced rodent AIMs was significantly reduced by the acute administration of compounds that have been shown to alleviate L-DOPA-induced dyskinesia both in parkinsonian patients and in rat and monkey models of Parkinson´s disease (amantadine, - 47%; buspirone, -46%; riluzole, -33%). The present data indicate that the mouse AIMs are indeed a functional equivalent of L-DOPA-induced dyskinesia.

Introduction

Dyskinesia (abnormal involuntary movements) is a common complication of L-DOPA pharmacotherapy in Parkinson´s disease (PD), affecting up to 80% of the patients within a few years from treatment onset (Obeso et al., 2000a). The most common form of dyskinesia consists in a combination of dystonic and choreiform movements occurring at the time when L-DOPA is providing the maximal relief of parkinsonian symptoms ("on" dyskinesias; (Marconi et al., 1994). Several lines of evidence concur to suggest that L-DOPA-induced dyskinesia results from a pulsatile stimulation of brain dopamine (DA) receptors, triggering a complex cascade of molecular and synaptic alterations within the basal ganglia (Chase, 1998; Bezard et al., 2001; Picconi et al., 2003). Knowledge of the plastic changes that prime the basal ganglia for a dyskinetic motor response to L-DOPA would be greatly advanced by the availability of a dyskinesia model in mice, which lend themselves to genetic manipulation of specific molecular and synaptic components. In a recent study (Lundblad *et al*., 2004) we have shown that unilaterally 6-hydroxydopamine (6-OHDA) lesioned mice treated with L-DOPA exhibit abnormal movements similar to those described in a validated model of L-DOPA-induced dyskinesia in the rat (for review see Cenci et al., 2002b). Mice that develop abnormal movements under L-DOPA show striatal upregulation of ΔFosB-like immunoreactivity and prodynorphin mRNA, two well-established markers of maladaptive molecular plasticity in other animal models of L-DOPA-induced dyskinesia (Doucet et al., 1996; Andersson et al., 1999). These results suggest that it is possible to simulate L-DOPA-induced dyskinesia in the mouse, but do not provide any information on the predictive validity and clinical relevance of the mouse dyskinesia model.

In this study, we compared the evolution of abnormal movements in 6-OHDA-lesioned mice treated chronically with either L-DOPA or other antiparkinsonian drugs that produce little or no dyskinesia when given "de novo". The two antiparkinsonian drugs used were the D2/D3 receptor agonist, ropinirole (Rascol et al., 2000), and the adenosine A2a receptor antagonist, KW-6002 (Kanda et al., 1998). Furthermore, we tested the effects of several substances that had been shown to reduce the severity of L-DOPA induced dyskinesia in both PD patients and rat and monkey models of PD. The substances tested here were the glutamate NMDA receptor antagonist, amantadine (Crosby et al., 2003); the glutamate release-inhibitor, riluzole (Merims et al., 1999) and Lundblad and Cenci, unpublished); the 5HT-1A receptor antagonist, buspirone (Bonifati et al., 1994) and Dekundy et al., 2003). The acute antidyskinetic effect of these drugs was tested in animals that had already been primed for dyskinesia. The mouse abnormal movements were found to respond to the drugs and treatments under investigation in the same way as classical L-DOPA-induced "on" dyskinesias. Thus, the present results provide pharmacological validation to the concept that the induction of dyskinetic movements by L-DOPA is a well conserved response from rodents to primates, although the physical manifestation of dyskinesia may have species-specific features.

Materials and methods

Subjects

Eighty-three male C57Bl/6 mice (B&K; 25 g body weight at the beginning of the study) were used in this study. The animals were housed under 12 hour ligth/12 hour dark cycle with ad libitum access to food and water. Housing conditions and treatment of the animals were in accordance with internationally accepted guidelines and were approved by the Malmö-Lund ethical committee on animal research (permit nb:195-02). All the mice used in this study sustained unilateral 6-OHDA injections in the striatum approx. 8 weeks prior to the initiation of any behavioural-pharmacological test.

Experimental design

The study comprised three experimental parts; *(1)* an initial experiment that aimed at comparing the antiakinetic effects of L-DOPA and different doses of ropinirole and KW-6002 in drug-naïve mice (n=12); *(2)* the main experiment (performed on a different group of mice; n=31), which aimed at assessing the evolution of abnormal movements in animals treated chronically with either L-DOPA, ropinirole, KW-6002, or vehicle. The chronic treatment was given for 21 days as indicated in Fig. 1; *(3)* finally, the L-DOPA-treated mice that had developed abnormal movements (n=12) were used to test the acute antidyskinetic effect of amantadine, buspirone and riluzole (Fig. 1).

Dose finding experiment for ropinirole and KW-6002

The study of ropinirole in 6-OHDA lesioned mice was preceded by a dose-response study of this compound in normal mice. The animals received i.p. injections of

ropinirole at the doses of 0.1, 0.5, and 1.0 mg/kg (n=3 per dose) and their levels of motor activation were then quantified using a videotrack monitor of horizontal activity. The highest dose of ropinirole was found to produce overt catalepsy, which was not seen with either of the two lower doses (although the intermediate dose tended to reduce the mouse activity counts; data not shown). The doses of 0.1 and 0.5 were therefore selected for evaluation in a test of forelimb akinesia (cylinder test) in drug-naïve, 6-OHDA lesioned mice (n=10). We have previously found that L-DOPA (at doses >6 mg/kg) significantly improves the forelimb asymmetry score in this test in the same lesion model. Both the high and the low dose of ropinirole were found to improve the performance of the impaired limb (+90% and +50%, respectively) compared to baseline (data not shown). Other groups of drug-naïve 6-OHDA lesioned mice (n=8) were tested with KW-6002 at the doses of 0.5 and 3 mg/kg. At the dose of 3 mg/kg, KW-6002 produced a significant improvement of forelimb use asymmetry in the cylinder test, which was greater than that obtained by the lower dose (79% vs 50% improvement respectively, data not shown). Taken together, these results suggested that 3 mg/kg KW-6002 and 0.1 mg/kg ropinirole were suitable doses to be compared with L-DOPA in the chronic drug treatment experiment.

Drugs and pharmacological treatments

L-DOPA (methyl-L-DOPA hydrochloride) and benserazide hydrochloride (a peripheral DOPA decarboxylase inhibitor) were purchased from Sigma Aldrich AB (Sweden). L-DOPA was freshly dissolved in physiological saline and injected i.p. in doses ranging from 20 to 30 mg/kg, combined with a fixed dose of 12 mg/kg benserazide (which was mixed in the same solution). Ropinirole hydrochloride (kindly provided by SmithKline Beecham Phamaceuticals, Worthing, UK) was

dissolved in physiological saline and given i.p. in doses ranging from 0.1 to 1 mg/kg. KW-6002 (kindly provided by H. Lundbeck Inc.) was dissolved by sonication in physiological saline containing 8% Tween-80 and given i.p. in doses ranging from 0.1 to 3 mg/kg. In the chronic drug treatment experiment, L-DOPA was given at the dose of 20 mg/kg/day (n=12); ropinirole at the dose of 0.1 mg/kg/day (n=7); and KW-6002 at the dose of 3 mg/kg/day (n=6). These drugs (or vehicle; n=6) were given in single daily i.p. injections for 21 days. In the last part of the study, we tested the acute effect of three substances that had been reported to reduce dyskinesia in PD patients and/or rat and nonhuman primate models of PD. The substances were administered in combination with a challenge dose of L-DOPA (30 mg/kg; i.p.; combined with 12 mg/kg benserazide). The general NMDA receptor antagonist, amantadine hydrochloride, and the glutamate release inhibitor, riluzole hydrochloride were purchased from Sigma Aldrich AB (Sweden). The partial 5-HT1A/D2 antagonist, buspirone was kindly provided by Merz Pharmaceuticals (Frankfurt; D). All these drugs were dissolved in physiological saline and given i.p. at the following doses in sequential tests, amantadine, 40 and 60 mg/kg; riluzole, 4 and 8 mg/kg; buspirone, 1 and 2 mg/kg. All the tested drugs except for amantadine were administered 30 minutes prior to L-DOPA. Amantadine was given 100 minutes before L-DOPA, because we previously found that this time interval confers maximal antidyskinetic potency to amantadine in the rat (Lundblad et al., 2002) and Dekundy and Cenci; unpublished). All drug solutions were prepared immediately prior to use and were injected in the volume of 10 ml/kg body weight.

Surgical procedures and assessment of 6-OHDA lesions

Eighty-three male mice (C57Bl/6) received unilateral 6-OHDA lesions in the right striatum using a procedure that has previously been found to produce > 75% reduction of DA fiber density (Lundblad *et al.* 2004). The surgical procedure was the same described in Lundblad *et al. (*2004) with one modification: instead of a burr hole, an incision was performed in the skull using the tip of an injection needle (∅ 1.25 mm). The mice were anaesthetized with Hypnorm®-Dormicum® (Apoteksbolaget, Sweden) (1:1:2 water mixture; 2.7 ml/kg body weight) and mounted in a stereotactic frame (Kopf Instruments; USA) with a mouse-adaptor, on a flat-skull position. 6-OHDA-HCl (Sigma Aldrich AB, Sweden) was dissolved in 0.02% ascorbate-saline at the concentration 3.0 µg freebase 6-OHDA per µl and injected into the striatum at the following coordinates (in mm) relative to bregma and the dural surface: (i) AP = +1.0, L = -2.1, DV = -2.9; (ii) AP = +0.3, L = -2.3, DV = -2.9 (Paxinos & Franklin, 2001)(2 injections x 2 µl). Each injection was performed at a rate of 0.5 µl/min using a glass capillary with an outer diameter of approximately 50 µm attached to a 10-µl Hamilton syringe. The injection cannula was left in place for additional 3 min before slowly retracting it.

Two weeks after the lesion, the mice were examined in a drug-free test of forelimb akinesia (cylinder test, see below). The cylinder test performed to screen the lesions prior to any drug treatment will be hereafter referred to as "baseline cylinder test". On this test, 64 mice were found to use the paw contralateral to the lesion in < 40% of all supporting wall contacts. This percentage of contralateral limb use corresponded to the mean minus two standard deviations of the values obtained in intact mice (Lundblad *et al.*, 2004), and was therefore regarded as a cut-off value for significant motor impairment. The ultimate assessment of lesion severity was

however carried out after completion of the behavioural studies by determining the striatal levels of tyrosine hydroxylase (TH) using Western immunoblotting . Two animals from the vehicle group, 3 animals from the L-DOPA group, 1 animal from the ropinirole group and 2 animals from the KW-6002 group were excluded from all statistical analysis because they showed a conspicuous (> 40%) sparing of striatal TH levels in the striatum.

Behavioural tests

The sequence of behavioural testing used in the main body of the study is illustrated in Fig. 1. During the chronic drug treatment experiment the mice were evaluated 7 times on the abnormal involuntary movements scale (i.e. on the first day of drug treatment and twice a week thereafter), and 3 times in the cylinder test (once a week). In the last part of the study (which aimed at testing the antidyskinetic effect of three compounds) L-DOPA-treated mice were evaluated in the abnormal involuntary movement scale on two consecutive days after the administration of L-DOPA, alone or in combination with one of the compound under investigation. This testing design was applied a total of 6 times (three drugs x two doses per drug) allowing at least 5 days interval between tests of different doses or drugs for drug washout. In all behavioural tests, the investigator was kept completely unaware of the animals´ treatment allocation (experimentally blinded).

Cylinder test

The cylinder test (Schallert & Tillerson, 2000) modified by (Lundblad et al., 2002) was used to monitor the antiakinetic effect of the various drugs. In short, each animal was placed in a glass cylinder (∅ 10 cm, height 14 cm) and was videotaped for 3 minutes. The mice responded to the novel environment by standing on their hindlimbs

and leaning on the walls of the cylinder with their forelimbs. The number of supporting paw-placements performed independently with the left and the right paw were counted. Only wall contacts where the animal supported its body weight on the paw with extended digits were counted. This criterion is essential to discriminate between accidental touches and meaningful physiological movements.
A lower limit of 5 wall contacts per session was set to ensure the sensitivity of the test. A limb use asymmetry score was computed by expressing the number of wall contacts performed with the forelimb contralateral to the lesion (left) as percentage of the total wall contacts.

AIMs ratings

Abnormal involuntary movements (AIMs) were scored using the rodent dyskinesia rating system described in Lundblad *et al.* (2004). Briefly, the animals were placed in separate cages and scored individually every 20 minutes (1-minute monitoring periods) after injection of the tested drugs. Scores were given to four different subtypes of (AIMs) namely axial, limb, orofacial and locomotive AIMs. Each of these subtypes was scored on a severity scale from 0 to 4 (0=no dyskinesia; 1= occasional dyskinesia displayed for < 50% of the observation time; 2=sustained dyskinesia displayed for >50% of the observation time; 3=continuous dyskinesia; 4=continuous dyskinesia not interruptible by outer stimuli). A description of the movements that are scored as dyskinetic (and those that are not) is given in Lundblad *et al.* 2004. From the raw data, an integrated AIM score was calculated as the area under the curve (AUC) in a plot of AIM score/monitoring period against total observation time (3 hours) (Winkler et al., 2002).

Activity monitor

In order to assess the general motor stimulant effects of different doses of ropinirole, KW-6002, or L-DOPA, horizontal motor activity was determined for three hours after drug administration using either a videotrack-based activity system (Viewpoint, Lyon, France) or infrared beam-based automated activity boxes (41 X 41 X 38 cm; flexfield with photobeam activity system for windows, version 2.0.5; San Diego Instruments Inc.). These tests were performed both in the pilot experiment that preceded the main body of the study, and at the end of the chronic drug treatment (Fig 1).

Determination of TH immunoreactivity by Western blotting

Mice were killed by decapitation, the striata were dissected out on an ice-cold surface, sonicated in 1% sodium dodecylsulfate and heated for 10 minutes. Aliquots (5µl) of the homogenate were used for protein determination using the BCA (bichinchoninic acid) assay kit (Pierce, Oud Beijerland, Netherlands). Thirty µg of protein from each sample were loaded onto polyacrylamide gels. The proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to polyvinylidine difluoride membranes (Amersham Pharmacia Biotech, Uppsala, Sweden), as described (Towbin et al., 1979). The membranes were immunoblotted using a polyclonal antibody against TH (a gift from Prof. Tomas Hökfelt, Karolinska Institutet, Stockholm, Sweden). Antibody binding was revealed by incubation with goat anti-rabbit horseradish peroxidase-linked IgG (Pierce, Netherlands) and the Enhanced Chemiluminescens Plus immunoblotting detection kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Chemiluminescence was detected by

autoradiography and immunoreactivities were quantified by densitometry, using the NIH Image software (version 1.61).

Statistical analysis

Integrated AIMs scores recorded in different testing sessions during the chronic treatment phase were analysed using repeated measures ANOVA, with treatment (L-DOPA, ropinirole, KW-6002 and vehicle) as the between-subjects variable. Post hoc comparisons were performed using Tukey test. Cylinder test data were analysed using either paired or unpaired t-test where appropriate. The effects of each antidyskinetic drug (and dose) on the mouse AIMs were analysed using Student's paired t-test, where the AIM scores obtained after the combined administration of the tested drug plus L-DOPA were compared to the scores recorded after the injection of L-DOPA and vehicle on the next (or preceding) day. Statistical significance level was set at $p<0.05$. Data are expressed as mean ± one SEM.

Results

Motor response to chronic treatment with L-DOPA, ropinirole or KW-6002

Thirty-one drug-naïve, 6-OHDA-lesioned mice were allotted to four groups to receive daily treatment with L-DOPA (20 mg/kg methyl L-DOPA combined with 12 mg/kg benserazide); ropinirole (0.1 mg/kg); KW-6002 (3 mg/kg); or vehicle for 21 days. The dose of L-DOPA used in this experiment was previously shown to improve performance in the cylinder test and to elicit dyskinetic-like movements in mice with the same type of lesion (Lundblad *et al.*, 2004).

Over the first two weeks of treatment there was a gradual increase in the incidence as well as severity of axial, limb and orolingual AIMs in the L-DOPA-treated animals ($p=0.001$ for treatment effect, $p=0.045$ for time effect), but not in any of the other groups ($p<0.0002$ for treatment and time interaction, repeated measures ANOVA, Fig 2A). Animals treated with ropinirole and KW-6002 did not display any of these dyskinetic behaviours. In fact, the L-DOPA treated group differed significantly from the other 3 groups in all testing sessions except for the first one (Fig. 2A). Unexpectedly, the L-DOPA-induced AIM scores tended to decline during the third week of treatment (Fig. 2A). We therefore carried out a closer analysis of the duration, incidence and peak-severity of L-DOPA-induced AIMs during the course of the experiment. In Fig. 2B, the L-DOPA induced AIM scores from each week of treatment are plotted against the time after a single L-DOPA injection. The resulting curves show that the time course of L-DOPA-induced mouse AIMs conformed to the temporal pattern of peak-dose dyskinesia (Marconi et al., 1994) throughout the course of the experiment. However, both the duration and the peak-severity of AIMs appeared to decline from the second to the third week of treatment. In order to

quantify these observations, we computed the duration of mouse AIMs using a formula similar to that described in studies of L-DOPA-induced rotation in 6-OHDA-lesioned rats (Papa et al., 1994). The duration of the L-DOPA-induced dyskinetic motor response, defined as the time during which the animals´ AIM scores exceeded 50% of their peak severity, was found to decrease significantly from the first to the third week of treatment ($p=0.047$, paired t-test,; Fig. 2C). The peak severity of dyskinesia (defined as the monitoring period where axial, limb, and orolingual AIMs reached their maximal values in each animal) showed a parallel, significant decline (Fig. 2D).

Interestingly, when repeated measures ANOVA was applied to the mouse locomotive AIM scores (Fig. 2E) there was no significant difference between the groups ($p=0.22$ for treatment effect; $p=0.51$ for treatment and time interaction), nor was there a significant overall change in locomotive AIMs during the course of the experiment ($p=0.66$ for time effect).

During the chronic treatment study, all the mice were evaluated in the cylinder test once a week. The three drugs under investigation produced an improvement of the mouse limb use asymmetry scores (Fig 3A). The percentage of supporting wall contacts performed with the paw contralateral to the lesion (left) was raised from 17 to 36% by ropinirole, and from 22 to 35% by KW-6002 ($p < 0.05$ vs. baseline performance for both drugs). L-DOPA enhanced the percentage left limb use from 22% to 62%, which represents an overshoot effect above normal performance (which amounts to ~ 50% left limb use; Lundblad et al., 2004). This overshoot effect should not be interpreted as an overcompensation of forelimb akinesia by L-DOPA, which we have never observed in previous studies (Lundblad et al., 2002; Picconi et al., 2003; Lundblad et al., 2004). The effect is instead related to the torsional movement

of the trunk towards the side contralateral to the lesion that was exhibited by the dyskinetic mice. This twisting movement seemed to prompt the animals to perform corrective steps with the left paw in order to maintain balance.

Horizontal activity

In order to verify whether the treatments under investigation had similar motor activating properties, automated recordings of horizontal activity were carried out in all mice on one occasion at the end of the chronic experiment (see filled circle in Fig 1). Activity counts did not differ significantly among mice treated with L-DOPA (20 mg/kg), ropinirole (0.1 mg/kg) and vehicle. KW-6002 (3 mg/kg) produced however a strong motor stimulant effect (Fig. 3B). The stimulation of horizontal activity by KW-6002 was dose-dependent, as it was not seen when KW-6002 was administered at 0.5 mg/kg (Fig. 3B). We then set out to exclude that the absence of dyskinetic manifestations in the KW-6002-treated group was due to a possible "masking effect" caused by increased locomotor activity. Two additional AIM rating sessions were carried out in this group of mice after the administration of 0.1 and 0.5 mg/kg KW-6002. These lower doses of the compound were found not to induce any behavioural signs of dyskinesia (data not shown).

Test of antidyskinetic drugs

The animals previously treated with L-DOPA were subjected to AIM ratings after an acute challenge with L-DOPA (30 mg/kg) and one of three non-dopaminergic drugs (amantadine, buspirone, and riluzole). The drugs and doses selected for this part of the study did not produce adverse motor effects in mice, as determined by a careful assessment of the literature and/or by preliminary testing of the compounds in small-scale pilot experiments (data not shown). Some of the treatments that are known to

reduce L-DOPA-induced dyskinesia in both PD patients and rat and monkey models of PD (e.g. clozapine) were not considered in this study because of their strong and generalized motor depressant effect in mice.

When coadministered with L-DOPA, the NMDA-receptor antagonist, amantadine reduced the mouse axial, limb and orolingual AIM scores by 30% and 50% at the doses of 40 and 60 mg/kg, respectively ($p=0.036$ and $p=0.0022$, paired t-test; Table 1). The 5-HT 1A receptor antagonist, buspirone, was tested at the doses of 1 and 2 mg/kg. The higher dose produced a significant, 45% reduction in axial, limb and orolingual AIM scores ($p=0.002$, paired t-test). The glutamate release inhibitor, riluzole was also tested at two different doses, namely 4 and 8 mg/kg. The lower dose produced a significant 33% reduction in the mouse AIM scores. The locomotive AIM scores were not significantly reduced by any of these treatments (Table 1).

Western immunoblotting for TH

In a previous study (Lundblad et al., 2004) this type of lesion was found to produce a reduction of DA fiber density by 65% and 75% in the medial and lateral striatum, respectively. In this study, the extent of the DA denervation was assessed using Western immunoblotting analysis of tyrosine hydroxylase (TH). All the animals included in the present study showed > 60% reduction of TH immunoreactivity in the 6-OHDA-lesioned striatum compared to the contralateral intact striatum. The levels of TH depletion did not show significant differences among the experimental groups (One-way ANOVA, followed by Newman-Keuls Multiple Comparison test) (Fig. 4).

Discussion

Rodent models of L-DOPA-induced dyskinesia provide an important and cost-effective tool for pathophysiological investigation and drug-screening experiments. A

rat model of L-DOPA-induced dyskinesia has been characterized and validated in a number of previous publications (reviewed in Cenci et al., 2002b; Cenci & Lundblad, 2005). In addition, we have reported the occurrence of abnormal movements and postures in unilaterally 6-OHDA lesioned mice treated chronically with L-DOPA (Lundblad et al., 2004). The mice exhibited torsion of the upper body and fluttering movements of the forelimb contralateral to the lesion, along with twitching and vacuous movements of the orofacial musculature. These abnormal movements could be reproducibly distinguished from naturally occurring behaviours. Compared to the rat dyskinesia model, the mouse AIMs were much more rapid, had a more simplified repertoire, and showed less prominent dystonic features (videofilms were published as supplementary data in Lundblad *et al*. 2004). The incidence of dyskinetic-like movements in mice was influenced by the extent of striatal DA denervation, and by the dose and duration of L-DOPA-treatment, all of which represent well-known risk factors for the development of L-DOPA-induced dyskinesia in both rat and nonhuman primate models of PD (Jenner, 2000; Winkler et al., 2002), and in the patients (Blanchet et al., 1996). Whereas a pharmacological validation of the rat dyskinesia model has been provided in a number of previous studies (Lundblad *et al*., 2002; Dekundy *et al*., 2003), it has thus far remained unknown whether the mouse AIMs have pharmacological features similar to L-DOPA-induced dyskinesia in PD. This question is highly warranted, because rodent species (and even strains of the same species) can exhibit pronounced differences in brain neurotransmitter activity and DA receptor-mediated control of motor behaviour (Puglisi-Allegra & Cabib, 1997; Sedelis et al., 2001).

The mouse axial, limb and orolingual AIMs show pharmacological features similar to L-DOPA-induced dyskinesia in rats and primates

In this study we have used a behavioural-pharmacological approach in order to verify the predictive validity of the mouse dyskinesia model. In one experiment, we have compared the effects of L-DOPA to those of antiparkinsonian medications that are devoid of dyskinesiogenic potential when given to nonhuman primates de novo. The evolution of AIMs was investigated over a 21-day period in different groups of mice that were treated with either L-DOPA, the D2/D3 DA receptor agonist ropinirole, or the adenosine A2a antagonist KW-6002. The three substances were given at daily doses that produced a significant amelioration of spontaneous forelimb use in the cylinder test. Treatment with L-DOPA rapidly induced dyskinetic movements of the trunk, limbs and orofacial region, which became more intense during the first two weeks of treatment. In contrast, ropinirole and KW-6002 produced no dyskinetic movements. Locomotive AIMs (which are an indirect measure of contralateral rotation) were also more pronounced in the mice treated with L-DOPA compared to the other two groups, but this group difference did not reach statistical significance. In the last part of the study, we tested the acute antidyskinetic efficacy of a number of compounds that have been reported to alleviate L-DOPA-induced dyskinesia in MPTP-intoxicated monkeys and/or PD patients. These experiments were carried out in the mice that had been rendered dyskinetic by a previous course of L-DOPA treatment. A randomized cross-over design was used to administer each compound or its corresponding vehicle in combination with L-DOPA prior to the rating of AIMs. The three compounds selected for this experiment (amantadine, riluzole, buspirone) proved to significantly reduce the mouse axial, limb and orolingual AIM scores. The same treatments did not however achieve a significant reduction of the locomotive

AIMs. In 6-OHDA-lesioned rats treated with L-DOPA, locomotive AIMs provide a poor predictor of dyskinesia since they may simply result from an asymmetric but otherwise normal locomotor activity (Lundblad et al., 2002). The present results indicate that, also in the mouse, locomotive AIMs provide a less sensitive behavioural measure of dyskinesia than do axial, limb and orolingual AIMs. Anatomical mapping of FosB/ΔFosB immunoreactivity has shown that axial, limb and orolingual AIMs are associated with an upregulation of this marker in the sensorimotor (lateral) part of the striatum, while locomotive AIMs are linked to increased ΔFosB expression in the medial striatum (Andersson et al., 1999; Lundblad et al., 2004). In both rats and mice, the medial striatum is involved with associative and limbic-related functions and plays a prominent role in the control of locomotor activities. In contrast, lateral (sensorimotor) domains are involved in the control of limb and orofacial movements, as well as DA-dependent stereotypies (Dickson et al., 1994; Puglisi-Allegra & Cabib, 1997). Interestingly, the sensorimotor part of the rodent striatum is the functional equivalent of the human putamen (Kish et al., 1988), which is the striatal region more affected by DA loss in the human disorder and the region showing alteration in opioid transmission (Piccini et al., 1997) and ΔFosB expression (Cenci et al., 2002a) in L-DOPA induced dyskinesia. These independent lines of evidence provide anatomical support to the contention that axial, limb and orolingual AIMs in rodents are indeed the functional equivalent of L-DOPA-induced dyskinesia in PD, while locomotive AIMs and contralateral rotation may provide a more general measure of behavioural activation.

Choice of lesion model

Intrastriatal injection of 6-OHDA was chosen as a model of PD in this study. In a previous work (Lundblad et al., 2004), we compared intrastriatal 6-OHDA lesions with complete 6-OHDA lesions of the medial forebrain bundle in the mouse. Intrastriatal 6-OHDA was found to yield a larger therapeutic window for L-DOPA than did the intramesencephalic injection, and offered two additional advantages, (i) it was associated with a much lower postoperative mortality rate; (ii) it reproduced more closely the topographical pattern of striatal DA depletion that is found in human PD. Although the 6-OHDA injection is made in the striatum, the ensuing DA-denervation extends also to extrastriatal structures that are believed to play an important role in the pathophysiology of dyskinesia (Obeso et al., 2004). Indeed, rodents do not exhibit an absolute segregation between striatal and extrastriatal DA projections within the basal ganglia motor circuitry. Two main types of axons have been identified within the rat nigrostriatal system. One axon type arborizes profusely in the striatum but sends also collaterals to globus pallidus, entopeduncular and subthalamic nuclei, while the other type of axon behaves conversely (Gauthier et al., 1999). Thus, intrastriatal 6-OHDA injections in rodents will cause retrograde degeneration of nigropallidal and nigro-subthalamic axons too. Accordingly, marked denervation of the globus pallidus has been documented in rats sustaining intrastriatal 6-OHDA lesions (Georgievska et al., 2002)

Motor fluctuations in the mouse

A common feature of "wearing-off" fluctuations in patients with PD is the shortening of the so-called "short duration response" to L-DOPA, which is defined both by the antiparkinsonian effect and by the "on phase" dyskinesia that follow a single

administration of L-DOPA (for review and discussion see Nutt & Holford, 1996; Obeso et al., 2000b). In unilaterally 6-OHDA lesioned rats a shortening of the rotational response to chronic L-DOPA has been proposed to model the wearing-off syndrome seen in humans (Papa et al., 1994; Marin et al., 2004). In this study we report for the first time that a similar effect occurs in mice. The mice in this study were given a constant dose of L-DOPA throughout the chronic drug treatment period, during which the AIMs duration decreased by approx. 30%. This effect was not observed in our previous study in mice (Lundblad et al., 2004), where L-DOPA was given chronically using a dose-escalating regime. The fact that the dose of L-DOPA was increased on consecutive treatment weeks precluded an observation of wearing-off type responses to the same doses. It is presently unclear whether dyskinesia and motor fluctuations share similar pathophysiological mechanisms. The possibility to model both types of motor complications in the mouse encourages further investigations on the molecular underpinnings of these adverse motor effects.

Unresolved issues

While the availability of a dyskinesia model in L-DOPA-treated mice represents a significant advance for future investigations, species differences in the control of motor behaviour should not been underestimated. An issue relevant to this study is the opposite behavioral response produced by high doses of dopamine D_2/D_3 receptor agonists (such as ropinirole) in the mouse, where they depress motor activity (Puglisi-Allegra & Cabib, 1997), and in the rat, where they stimulate motor activity (Prikhojan et al., 2000). This discrepancy is most likely due to the fact that in the mouse D2-like agonists act preferentially at the presynaptic level, thereby reducing DA release via activation of inhibitory autoreceptors (for review see Puglisi-Allegra & Cabib, 1997).

In the present experiments ropinirole was used at a low dose that had no motor depressant effect and produced a significant improvement of parkinsonian-like motor features (i.e. deficit in spontaneous forelimb use). However, ropinirole was clearly less effective than L-DOPA in terms of antiakinetic potency, probably due to its more limited action at the postsynaptic level. This issue should be taken into account in future studies that aim at screening dopamine D_2/D_3 receptors agonists in 6-OHDA-lesioned mice.

Another issue that remains unresolved in the decrease in AIM score that the mice exhibited during the last week of chronic L-DOPA treatment. A closer analysis of the data revealed that a shortening in the duration of the motor response to L-DOPA may have contributed to this phenomenon. This type of motor response alteration has been previously reported in unilaterally 6-OHDA lesioned rats treated with L-DOPA (Papa et al., 1994), and has been suggested to provide an animal model of wearing-off fluctuations in PD (Marin et al., 2004). However, the shortened response did not fully account for the conspicuous decrease in AIM score during the last week of L-DOPA treatment. From the second to the third week of treatment, the peak severity of dyskinesia had declined too. The finding of declining AIM severity upon repeated treatment with L-DOPA was unexpected, and had not been previously encountered in 6-OHDA lesioned rats. This phenomenon is unlikely to depend on a loss of post-synaptic neurons. Indeed, total levels of the striatum-specific protein DARPP-32 were not decreased in any of the experimental groups (data not shown). The possibility of spontaneous recovery in the lesioned nigrostriatal system was excluded by measuring striatal TH levels. Some of the animals showing the most dramatic decrease in dyskinesia severity over time were found to exhibit the most pronounced depletion of striatal TH levels (1-2 % of normal). The observed decline in

AIM severity during the course of chronic L-DOPA treatment is not possible to explain on the basis of the results obtained in this study, but calls for future investigations. A hypothesis that is presently close at hand is one of a densitization of brain D1 receptor upon repeated administration of L-DOPA. In 6-OHDA lesioned rats, desensitization of rotational responses is seen after treatment with D1- but not D2 receptor agonists (Asin et al., 1995), It is conceivable that the motor stimulant properties of L-DOPA in the mouse are dependent on postsynaptic D1 receptors to a larger extent than they are in the rat (Puglisi-Allegra & Cabib, 1997).

Acknowledgements

The authors warmly thank Dr. Andrzej Dekundy (Merz Pharmaceuticals, Frankfurt, Germany) for supplying buspirone for this experiments, and for his very helpful comments. This study was supported by grants from the M. J. Fox Foundation for Parkinson´s Research (M.A.C. and G.F.), from the Swedish National Research Council (M.A.C. and G.F.), and from the Konung Gustaf V:s Drottning Victorias Foundation (M. A. C.). A. U. was supported by a fellowship from the Wenner-Gren Foundations. M. C. was supported by a Marie Curie Host Training Fellowship.

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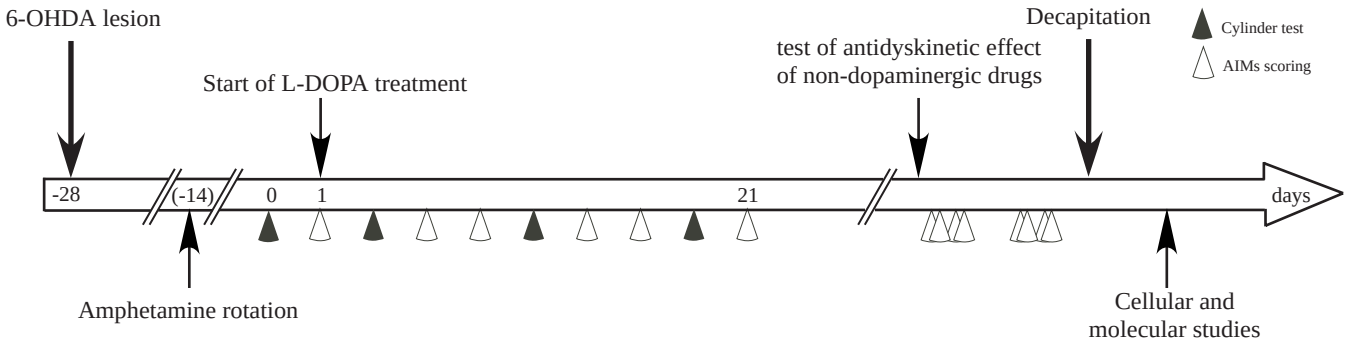
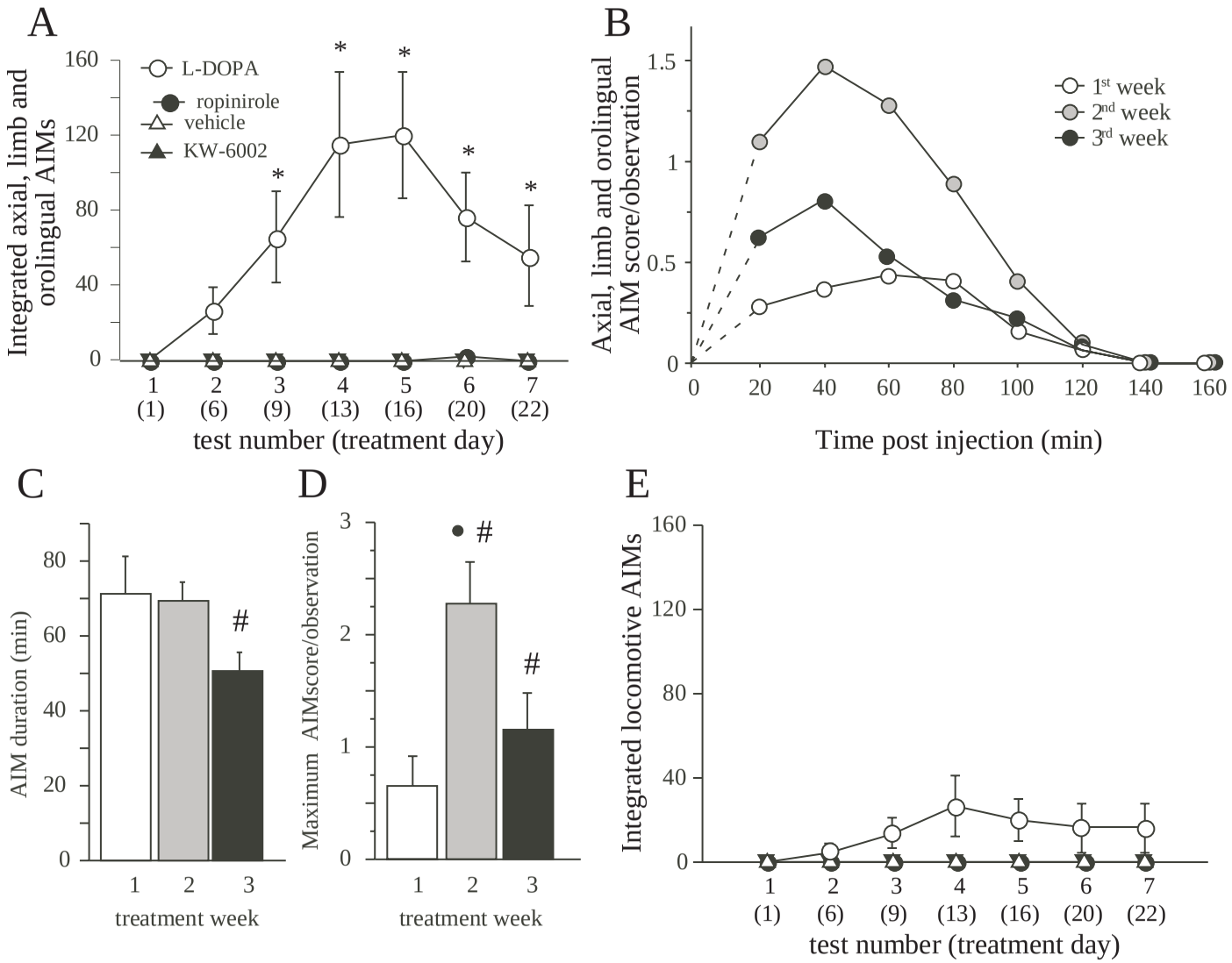
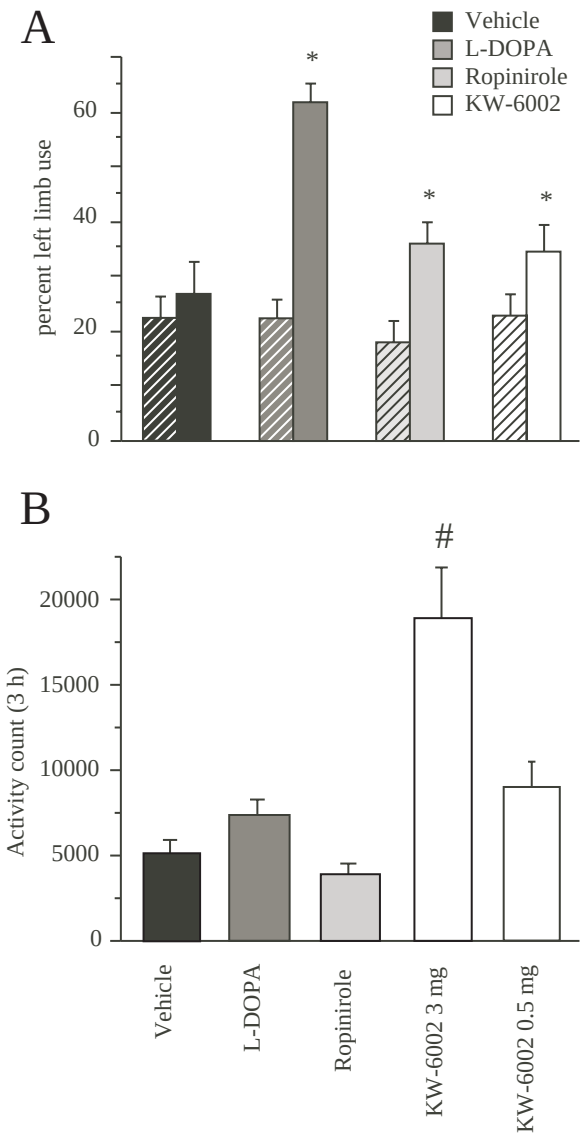
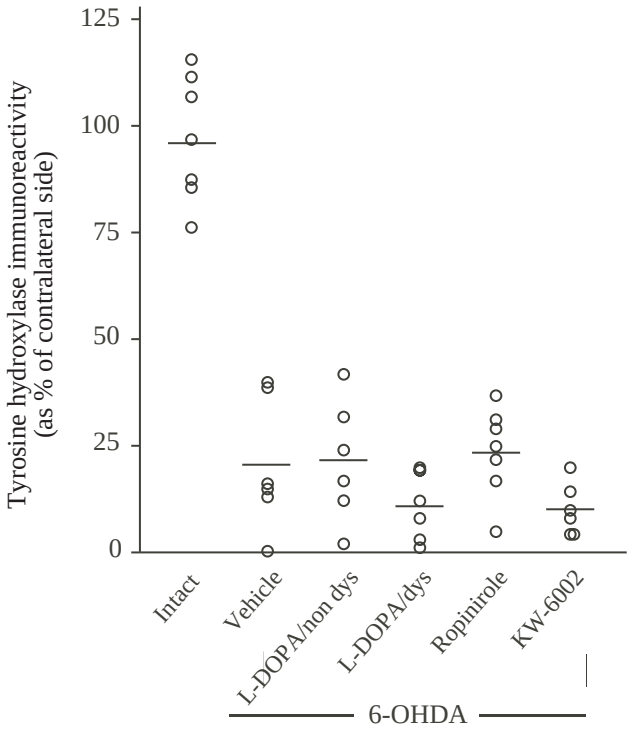

6-OHDA lesion
Start of L-DOPA treatment
test of antidyskinetic effect
of non-dopaminergic drugs
Decapitation
Cylinder test
AIMs scoring
-28
(-14)
0
1
21
days
Amphetamine rotation
Cellular and
molecular studies


A
Integrated axial, limb and orolingual AIMs
L-DOPA
ropinirole
vehicle
KW-6002
test number (treatment day)
B
Axial, limb and orolingual AIM score/observation
1st week
2nd week
3rd week
Time post injection (min)
C
AIM duration (min)
treatment week
D
Maximum AIMscore/observation
treatment week
E
Integrated locomotive AIMs
test number (treatment day)


A
Vehicle
L-DOPA
Ropinirole
KW-6002
percent left limb use
0
20
40
60
*
*
*
B
Activity count (3 h)
0
5000
10000
15000
20000
#
Vehicle
L-DOPA
Ropinirole
KW-6002 3 mg
KW-6002 0.5 mg


Tyrosine hydroxylase immunoreactivity
(as % of contralateral side)
125
100
75
50
25
0
Intact
Vehicle
L-DOPA/non dys
L-DOPA/dys
Ropinirole
KW-6002
6-OHDA

	AIM score change (%)	
Compound	**ALO**	**Lo**
Amantadine		
40 mg/kg	-28.7 ±19.6% *	-11.3 ±27.8%
60 mg/kg	-47.6 ±14.6% *	-60.0 ±20.5%
Buspirone		
2 mg/kg	-45.7 ±18.4% *	-34.1 ±28.9%
Riluzole		
4 mg/kg	-33.1 ±22.6% *	-6.9 ±36.6%