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Research report

Cabergoline and pramipexole fail to modify already established dyskinesias in an animal model of parkinsonism

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ABSTRACT

Levodopa-induced dyskinesias are one of the major limiting side effects encountered in the treatment of Parkinson's disease. Dopamine agonists of the D2 family are less prone to induce these abnormal involuntary movements (AIMs), and in some instances it has been proposed that they could counteract them once already established. As differences in the plasma half-life of a given DA agonist could be related with a greater or lesser propensity to induce or to counteract AIMs, we compared the effects of two D2 agonists (cabergoline and pramipexole) with different half-lives, and levodopa, at doses producing similar improvement in purposeful forelimb use, in rats with severe nigrostriatal lesion, previously sensitized to levodopa. The same therapeutic regime was subsequently used in pharmacologically naïve rats. We found that: (i) prior induction of AIMs by levodopa administration primes rats for the occurrence of AIMs during mono-therapy with pramipexole (but not with cabergoline); (ii) an intervening period of D2 agonist mono-therapy does not modify the severity of AIMs induced by subsequent mono-therapy with levodopa; iii. *de novo* treatment with D2 agonists is associated with a lower risk of AIMs (regardless of the severity of the lesion) and does not modify AIMs during subsequent mono-therapy with levodopa. An unexpected finding was that prior levodopa therapy sensitized rats to the therapeutic effects of D2 agonists given in mono-therapy. In summary, the use of the rat with nigrostriatal lesion to model relevant therapeutic conditions does not support that D2 agonists prevent the development of AIMs during subsequent levodopa mono-therapy or can revert the dysfunction underlying it.

1. Introduction

A common and troublesome complication of levodopa therapy in Parkinson's disease (PD) is the development of abnormal involuntary movements (AIMs) commonly designated as levodopa-induced dyskinesias (LID), and present preferentially during the peak effect of the drug (peak dose dyskinesias). LID may stem from pulsatile stimulation of striatal dopamine receptors [3,25]. Indeed, it is believed that a more constant activation of dopamine receptors might counteract LID [21,23]. However, the pharmacological factors determining the risk of experiencing AIMs remain poorly understood, and how to prevent them is still a matter for debate.

De novo mono-therapy with selective D2-family dopamine receptor agonists is associated with a lower risk of dyskinesias, both in non-human parkinsonian primates [11,12,19,31] and in PD patients [5,14,33,34]. However, this is perhaps achieved at the expense of a reduced therapeutic benefit [5,14,34]. Moreover, the previously accepted notion that *de novo* D2 agonist mono-therapy could delay the development of dyskinesias during subsequent levodopa therapy is under review [7,36]. Patients with less aggressive forms of PD could perhaps remain under D2 agonist mono-therapy for longer, making it difficult to establish whether the reduced risk of dyskinesias is related to D2 selectivity or to different rates of disease progression. On the other hand, if LID have been previously established, D2 agonist mono-therapy is likely to evoke its reappearance in a way similar to levodopa [4,18,22,30,34]. An exception could be the long-acting D2 agonist cabergoline, which has been reported as being not only devoid of AIMs inducing effects but also as capable of counteracting LID in parkinsonian monkeys rendered dyskinetic by chronic levodopa [1,13]. Despite its significantly longer half-life, cabergoline has not shown, in clinical practice, superiority to shorter acting compounds both in preventing newly developed dyskinesia or in reducing it once it has already developed [5,27,28,34]. It remains unclear whether propensity to experience dyskinesias during D2 agonist mono-therapy, at therapeutically effective doses, in individuals with similar extents of

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nigrostriatal damage, depends on prior priming by non-selective dopamine receptor stimulation. Moreover, there is little evidence that D2 agonist mono-therapy influences the probability of developing LID or can reverse already established LID [23].

Here, we compared the ability of cabergoline and pramipexole, two D2 agonists that are currently in use in the clinical setting, with that of levodopa, to induce AIMs, at doses producing similar therapeutic benefit, in rats with severe nigrostriatal degeneration. Cabergoline binds steadily to D2-family receptors for >72 h and has a plasma half-life of about 90 h [10]. Pramipexole has full intrinsic activity on D2-family receptors and has a plasma half-life of 7–9 h [2,24]. Therapeutic benefit was determined by assessing the effect of drug treatments on spontaneous purposeful forelimb movements, as in rodents nigrostriatal degeneration causes a forelimb use deficit that may be analogous to the upper extremity bradykinesia occurring in PD [37]. Additionally, we evaluated the relative abilities of cabergoline and pramipexole to prevent or counteract LID.

2. Materials and methods

2.1. Animals

Female Wistar rats ($n = 60$, 200–220 g) purchased from Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (Buenos Aires, Argentina) were caged in groups of four, with free access to food and tap water in a temperature-controlled room (21 °C) with a 12 h light/dark cycle, and cared for, in accordance with the NIH guide for the Care and Use of Laboratory Animals, as well as Argentine regulations (RS617/2002, Servicio Nacional de Sanidad y Calidad Agroalimentaria).

2.2. Drugs

Desipramine hydrochloride (Sigma, St. Louis, MO, USA), ketamine (Holliday-Scott), xylazine (Kensol, König), 6-hydroxydopamine hydrobromide (6-OHDA-HBr, Sigma, St. Louis, MO, USA), amphetamine, apomorphine (Sigma, St. Louis, MO, USA).

Commercially available Levodopa/carbidopa 250/25 mg (Lebocar, Pfizer), Cabergoline 2 mg (Cabaser, Pfizer), Pramipexole 1 mg (Sifrol, Boehringer-Ingelheim) tablets were dissolved in tap water. Intragastric administration of levodopa and of the D2 agonists was performed at 5–6 pm except the days of behavioural evaluation, which required treating the rats in groups of 6–8 between 10 a.m. and 4 p.m.

2.3. Severe unilateral 6-OHDA lesion

In order to obtain an extensive unilateral nigrostriatal degeneration, surgery was performed following a published protocol [8]. Under deep anesthesia (ketamine/xylazine 40/10 mg/kg, i.p. plus lidocaine at pressure points) rats received a stereotaxic injection of 8 μg/4 μl of 6-hydroxydopamine (6-OHDA, free base, 0.55 μl/min) dissolved in distilled water containing 0.1% of ascorbic acid. To prevent uptake by noradrenergic neurons, animals were pretreated with desipramine (25 mg/kg, i.p.) 30–45 min before injection of 6-OHDA. The injection site was the left medial forebrain bundle, stereotaxic coordinates from bregma (mm): 2.8 posterior, 2 lateral and 8.6 ventral [29]. Rats were placed on a heating pad to minimize hypothermia until they recovered from anesthesia.

2.4. Tyrosine Hydroxilase immunohistochemistry

Two hours after the last drug administration (and after behaviour evaluation), rats were anesthetized with ketamine/xylazine (40/10 mg/kg, i.p.) and perfused transcardially with 100 ml of saline followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were post-fixed for 2 h in the same fixative solution, cryoprotected in 30% sucrose in 0.1 M PBS for 48 h, frozen in isopentane at −35 °C and stored at −70 °C until sectioning. Serial coronal, 40-μm-thick tissue sections of striatum and *substantia nigra pars compacta* (SNpc) were cut in a freezing microtome. The slices were stored in PBS containing 0.1% sodium azide at 4 °C. Immunohistochemistry was carried out in free-floating sections. They were rinsed with 0.1 M PBS containing 0.15% Triton (PBS-T) and incubated for 1 h with PBS-T containing 0.3% H_2O_2 and 50% methanol for inhibition of endogenous peroxidase. After washing with PBS-T, non-specific binding of immunoreagents was blocked by 1 h incubation in 10% normal goat serum in PBS-T. Sections were incubated overnight at 4 °C with rabbit anti-tyrosine hydroxilase antiserum (TH, 1:1000, Peel Freeze). Three rinses with PBS-T were performed before incubating with biotinylated anti-rabbit IgG (1:250, Vector Laboratories) for 2 h. The antibody–antigen complex was visualized by means of an avidin–biotin peroxidase complex (1:125, ABC kit, Vector Laboratories), developed with one tablet of 10 mg of 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA) and 3 μl H_2O_2 in 20 ml Tris buffer 0.25 M.

2.5. Behaviour

Akinesia of the contralateral forepaw was assessed in a limb-use asymmetry test (cylinder test) [37]. Briefly, a rat is placed in a transparent acrylic cylinder (20 cm diameter, 30 cm height) and the observer counts the number of wall contacts performed with the left, right, or both forelimbs simultaneously, during 5 min of spontaneous vertical exploration. An asymmetry score was calculated as the number of contralateral forelimb wall contacts plus 1/2 the number of both forelimbs wall contacts, divided by the total number of wall contacts (ipsilateral plus contralateral plus both forelimb contacts) [40]. The limb-use asymmetry test was performed before and after surgery and 1 h after drug administration.

Turning behaviour was recorded in automatic rotameters. Briefly, rats were placed in the rotameters and the difference between the number of full contralateral and ipsitateral turns was registered every 5 min for 1 h in the case of amphetamine and apomorphine, and for 2 h after levodopa and dopamine agonists administration. Peak turning activity is the average number of turns per min during the 20 min centered on the 5 min of maximal net turns.

Two types of AIMs were rated separately, forelimb dyskinesia (FD) and axial dystonia (AD), according to the following scale: 0 = absent; 1 = occasional; 2 = frequent; 3 = continuous interrupted by sensory distraction; 4 = continuous not interrupted by sensory distraction [6,8,16]. Masticatory dyskinesias (MD) scores are not reported here, because, as described before [8], observers can easily rate normal orolingual movements as MD. Moreover, in our experience both FD and AD have a superior discriminating power to indicate the presence of dyskinesia than MD [8]. AIMs were evaluated during 2 min every 30 min for 2 h after a drug challenge. As no treatment had a preferential effect on a given type of AIM (not shown), the maximal scores of FD and AD recorded after a drug challenge were added, given a single AIM score per rat per drug challenge with values ranging from 0 to 8.

2.6. Experiment 1: Behavioural effects of D2 agonists after sensitization with levodopa

Two weeks after 6-OHDA injections, rats were assessed for turning behaviour induced by amphetamine (1 mg/kg, i.p.), and a week later, by apomorphine (0.25 mg/kg, s.c.). Rats that performed more than 100 net ipsilateral turns after amphetamine and 100 net contralateral turns after apomorphine in 1 h were selected for the experiment ($n = 39$). Selection of the rats by pharmacological challenge in this experiment was believed to have no impact on the outcome as all animals were to be initially primed to the effects of levodopa. Rats were also assessed in the cylinder test the day before surgery, two weeks after surgery and again the day before initiation of chronic drug treatment. Since day 28 post-surgery the rats were randomly divided into four groups and for 10 consecutive days (Fig. 1A), all rats received an oral dose of 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation). Then, the rats received for the next 10 days an oral daily dose of: (a) vehicle (tap water, $n = 9$), (b) 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation) ($n = 9$), (c) 2 mg/kg cabergoline ($n = 10$), (d) 1 mg/kg pramipexole ($n = 11$). After a wash out of 10 days, all rats received a second 10-day course of oral daily levodopa 50 mg/kg (corresponding to 250/25 levodopa/carbidopa formulation). Turning behaviour and AIMs were assessed on drug treatment days 1, 5 and 10, limb-use asymmetry on drug treatment days 3 and 8. The dose of levodopa was that which, in a dose response finding experiment (data not shown), produced significant functional recovery (of forelimb use) and significant amount of dyskinesia. Doses of cabergoline and pramipexole were those that matched levodopa in its ability to improve forelimb use.

2.7. Experiment 2: Behavioural effects of D2 agonists when administered de novo

Drug-naïve 6-OHDA-lesioned rats ($n = 21$) were evaluated in the cylinder test the day before and two weeks after the 6-OHDA injections. They were also assessed in the cylinder test the day before initiation of chronic drug treatment. Rats were randomly divided into three groups at day 28 after 6-OHDA injection and received oral daily doses of (a) 50 mg/kg levodopa ($n = 8$) (corresponding to 250/25 levodopa/carbidopa formulation); (b) 2, 3, and 4 mg/kg cabergoline ($n = 7$); (d) 1, 1.5, and 2 mg/kg pramipexole ($n = 6$), at the different stages of the experiment (Fig. 1B). The dose of levodopa was the same as in the previous experiment and it was maintained stable during the whole experiment. Starting doses of both DA agonists were those used in the previous experiment, which in sensitized animals were able to produce comparable functional improvement to levodopa. These doses had to be subsequently titrated upward as they were initially unable to produce a degree of functional recovery comparable to levodopa. During the first 10 days, the first group received levodopa 50 mg/kg (corresponding to 250/25 levodopa/carbidopa formulation), while the other two groups received 2 mg/kg cabergoline and 1 mg/kg pramipexole respectively. In the second phase of the experiment the first group receiving levodopa was maintained in the same regimen. The other two groups received increasing doses of D2 selective agonists, first, 3 mg/kg cabergoline and 1.5 mg/kg pramipexole for 5 days, and then 4 mg/kg cabergoline and 2 mg/kg pramipexole for the remaining 5 days. The dose of cabergoline was not increased beyond 4 mg/kg, as the animals developed ipsilateral rotational behaviour without any evidence of reversal in the cylinder test. Turning behaviour, AIMs and limb-

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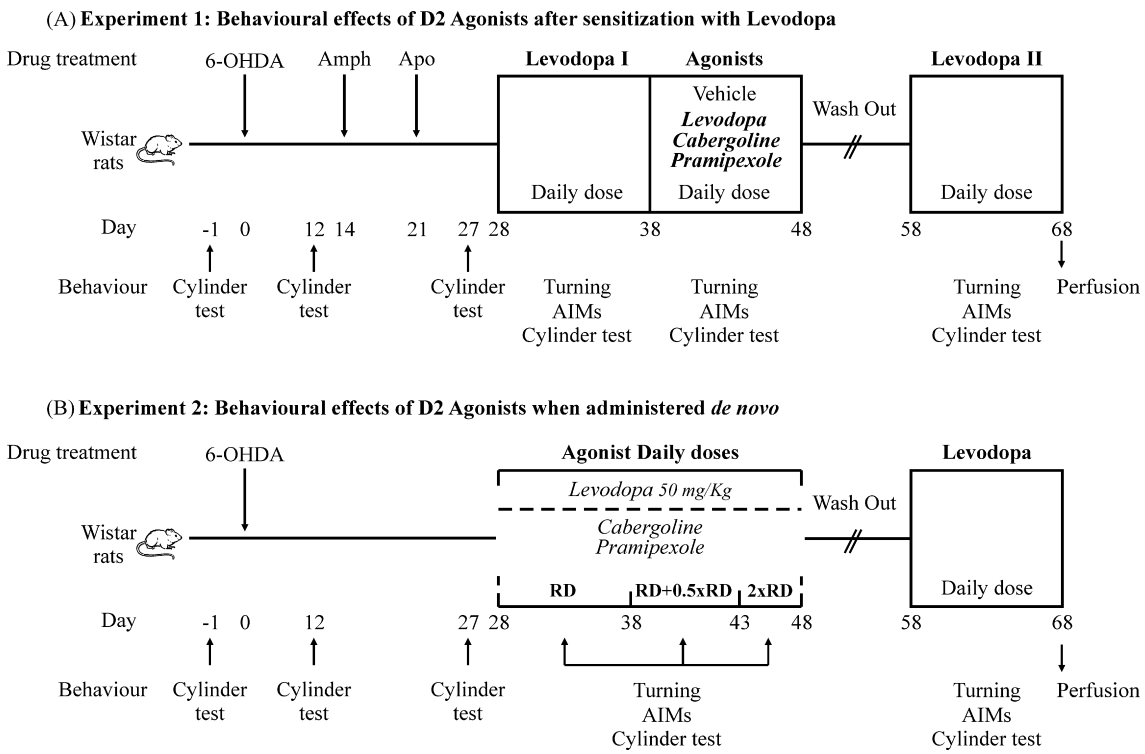

Fig. 1. Schematic summary of experiments, experimental groups and drug treatments (see text for details). 6-OHDA: 6-hydroxydopamine, Amph: amphetamine, Apo: apomorphine, AIMs: abnormal involuntary movements, RD: reference dose.

use asymmetry were assessed as described above. In both instances, we measured AIMs and turning behaviour on the 5th administration day and performance in the cylinder test on the 3rd administration day. To assess the effect of *de novo* exposure to D2 agonists on the chances of provoking LID, following a 10 day wash out, the three rat groups were treated for 10 days with 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation) and evaluated for AIMs on days 1, 5 and 10.

2.8. Statistical analysis

Turning rates are reported as average contralateral minus ipsilateral turns per minute ± S.E.M. during the 20 min of maximal activity. Performance in the cylinder test is reported as average asymmetry score (see above) ± S.E.M. An asymmetry score of 50% (*dotted lines* in graphs) indicates equal probabilities for the use of the ipsilateral and contralateral forepaws, 0%, no use of the contralateral forepaw. Comparisons of turning rates and cylinder test scores were performed by means of one-way analysis of variance (ANOVA) followed, when significant differences existed, by the *post hoc* Tukey test for unequal number of observations. As AIM scores did not fit a normal distribution, data were summarized as median scores of the sum of axial dystonia and forelimb dyskinesia (*small white boxes*, maximal possible score = 8) and ranges (*large boxes*, 25–75% interquartile; *error bars*, range). Comparisons involving AIM scores were done with a Kruskal–Wallis ANOVA followed by the Dunn's multiple comparisons test.

3. Results

Post mortem immunohistochemistry confirmed an almost complete depletion of TH positive (TH+) cell bodies in the SNpc (<10 TH+ cells per coronal mesencephalic section) and of TH+ axon terminals in the striatum ipsilateral to the 6-OHDA injection site in all rats included in the present study (Fig. 2A, microphotographs of brain sections from a representative rat).

3.1. Experiment 1: Behavioural effects of D2 agonists after sensitization with levodopa

Two to three weeks post surgery, rats ($n = 39$) with unilateral severe damage to the nigrostriatal pathway displayed strong ipsilateral turning behaviour in response to amphetamine and contralateral turning behaviour in response to apomorphine (Fig. 2B), and a marked deficit of contralateral forepaw use in the cylinder test (Fig. 2C; $t_{36} = 12.91$, $^{**}p < 0.01$ *t*-test for dependent samples).

3.2. First oral daily levodopa treatment

The rats ($n = 39$) were randomly divided into four experimental groups and assessed for the effect of oral daily levodopa on turning behaviour, AIMs and contralateral forelimb use in the cylinder test (Fig. 3A–C). Levodopa, administered for 10 consecutive days, induced contralateral turning behaviour and marked AIMs in all rats both at days 5 and 10 of treatment. No differences were observed between recordings taken on days 5 and 10 (not shown), therefore, data were averaged (Fig. 3A and C). Due to the severity of levodopa-induced AIMs, some rats could not be reliably assessed in the cylinder test (14 out of 39). Those which could, showed a recovery of contralateral forelimb function, or even a preferential use of the contralateral limb (Fig. 3B). The four experimental groups did not differ in any behavioural measure (turning behaviour: one-way ANOVA, $p = 0.971$; cylinder test: one-way ANOVA, $p = 0.276$; AIMs: Kruskal–Wallis ANOVA, $p = 0.703$). These results confirm previous findings showing that levodopa administration to 6-OHDA-lesioned rats reverses a deficit of spontaneous motor activity resembling akinesia and induces AIMs similar to levodopa-induced peak dose dyskinesias [17].

3.3. Treatment with dopamine agonists after sensitization to levodopa

The main aim of this study was to determine whether the likelihood of showing dyskinesias during chronic treatment with "therapeutically effective" doses of D2 family agonists depends on the plasma half-life of the drugs. The D2 agonists cabergoline and

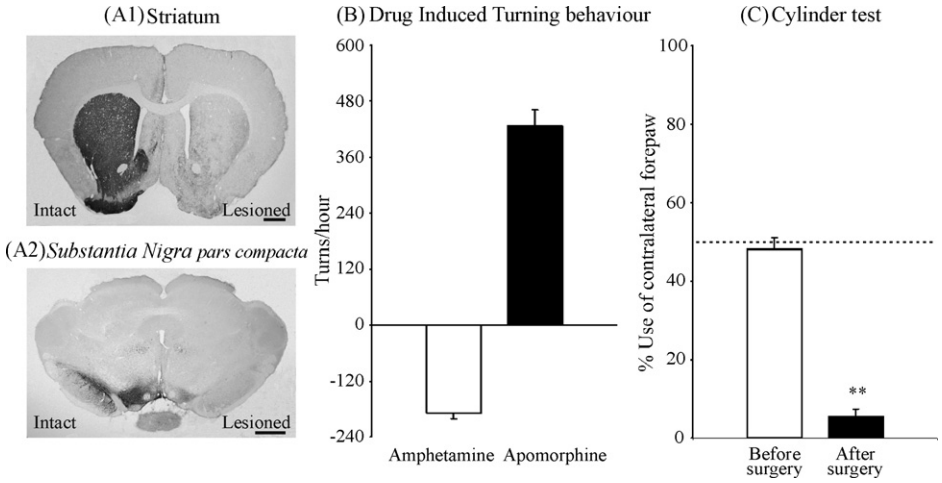

Fig. 2. Characterization of the 6-OHDA-induced lesion. (A) Representative striatal (A1) and mesencephalic (A2) sections immunostained with an antiserum against tyrosine hydroxylase. Scale bars: 100 μm. Drug induced turning behaviour (B) and performance in the cylinder test (C) of rats with nigrostriatal lesion (mean ± S.E.M., $n = 39$, $^{**}p < 0.01$). The *dotted line* indicates symmetric performance.

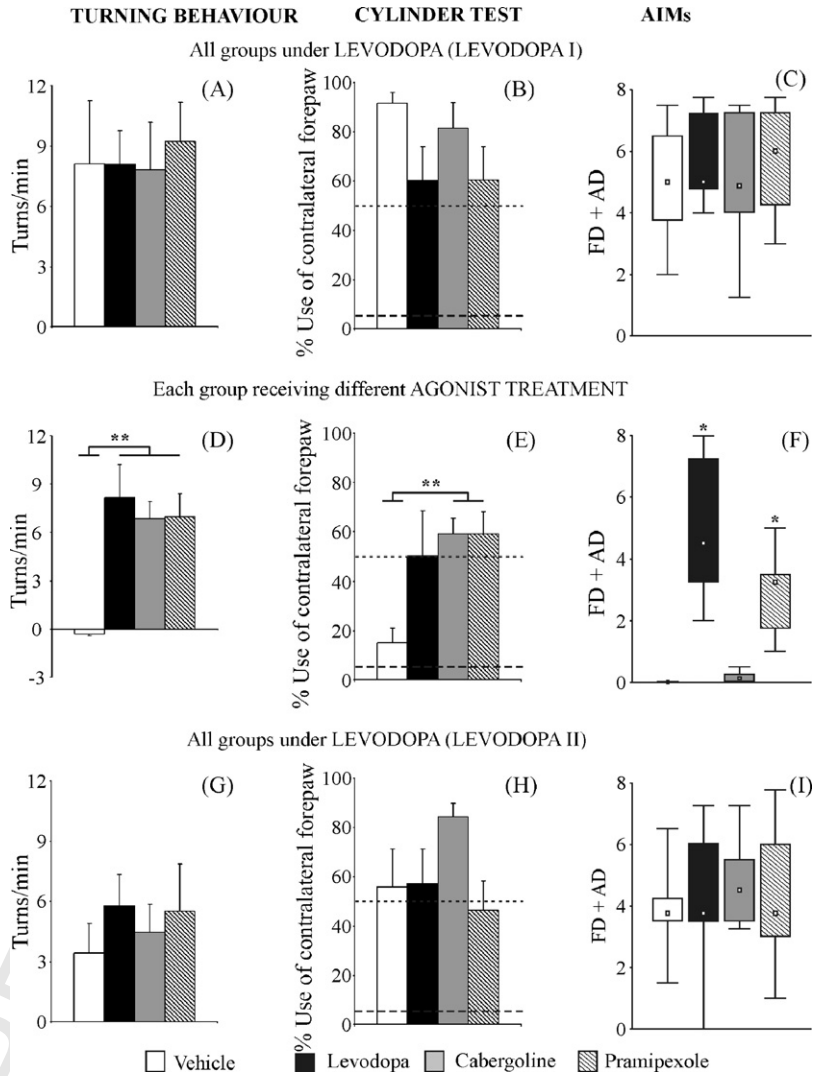

Fig. 3. Experiment 1: Behavioural effects of D2 agonists after sensitization to levodopa. Turning behaviour (A, D, G), performance in the cylinder test (B, E, H) and AIMs (C, F, I) recorded in four rat groups during a 10 day treatment with levodopa (A, B, C), during subsequent treatment of the same animals for 10 days with vehicle, pramipexole, cabergoline or levodopa (D, E, F), and next during a similar treatment with levodopa (G, H, I). $^{*}p < 0.05$; $^{**}p < 0.01$, *post hoc* comparisons after significant main effects in ANOVAs. The *dashed line* is the forelimb performance recorded in the whole set of rats after 6-OHDA-induced lesions, in a drug free condition before starting the drug treatments.

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pramipexole, at the doses of 2 mg/kg and 1 mg/kg, respectively, were as efficacious as levodopa in improving contralateral forelimb performance in the cylinder test (Fig. 3E; one-way ANOVA, $F_{(3,31)} = 5.88$, $p = 0.003$; $^{**}p < 0.01$ versus vehicle, Tukey test for unequal *N post-hoc* comparisons). Note that, as some rats showed marked AIMs under levodopa and could not be reliably evaluated in the cylinder test, the number of observations was too small in the group under levodopa treatment and the *post hoc* comparison did not reach significance for levodopa versus vehicle in Fig. 3E. Moreover, the D2 agonists were equipotent in their ability to induce contralateral turning behaviour when compared with levodopa (Fig. 3D; one-way ANOVA, $F_{(3,35)} = 7.43$, $p = 0.0006$; $^{**}p < 0.01$ versus vehicle). However, cabergoline-induced rotations differed from those induced by pramipexole and levodopa. Cabergoline induced constant contralateral turning without the typical torsion of the head and body due to axial dystonia that is observed during levodopa- or pramipexole-induced turning behaviour (not shown). Moreover, cabergoline did not induce AIMs (median score: 0.13), whereas pramipexole and levodopa induced severe AIMs (median score: 3.25 and 4.5, respectively) (Fig. 3F; Kruskal–Wallis ANOVA, $H_{(3,39)} = 32$, $p < 0.0001$; $^{*}p < 0.05$ levodopa or pramipexole versus vehicle or cabergoline). Overall, these results indicate that, in rats rendered dyskinetic by chronic levodopa, the likelihood of inducing dyskinesias during a subsequent therapeutically effective D2 agonist chronic treatment does depend on pharmacological characteristics of D2 agonists.

3.4. Second treatment with levodopa

The second aim of this study was to determine whether monotherapy with a D2 agonist of rats that were first rendered dyskinetic by levodopa administration can reduce the likelihood of showing dyskinesia during a subsequent treatment with levodopa. As the plasma half-life of cabergoline in rats is about 65–110 h [10], a wash out of 10 days was allowed before starting a second levodopa treatment in all rats, at the same dose which was effective in inducing AIMs at the beginning of the experiment. During this second treatment, levodopa was equally effective in inducing contralateral turning behaviour, contralateral forelimb use and AIMs, (turning behaviour: one-way ANOVA, $p = 0.800$, for contralateral forepaw use: one-way ANOVA, $p = 0.169$; AIMs: Kruskal–Wallis ANOVA, $p = 0.802$) regardless of the pharmacological treatment received by

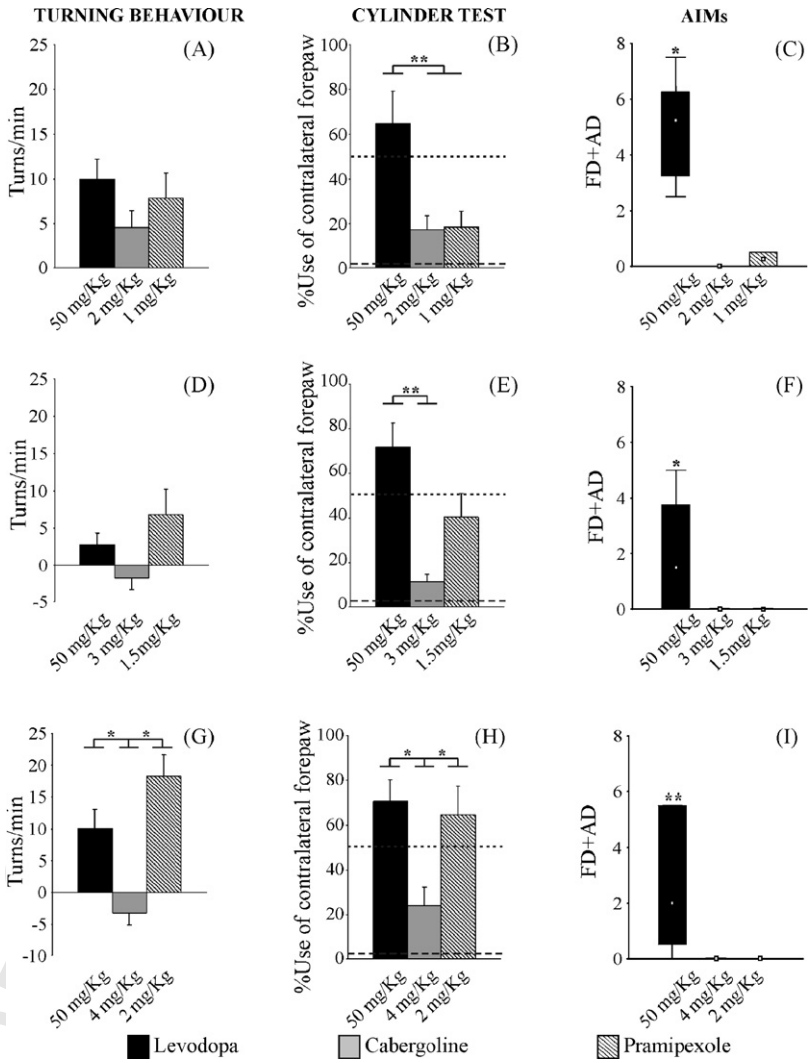

Fig. 4. Experiment 2: Behavioural effects of D2 agonists when administered *de novo*. Turning behaviour (A, D, G), performance in the cylinder test (B, E, H) and AIMs (C, F, I) recorded in three rat groups during a chronic treatment with 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation), or increasing doses of pramipexole and cabergoline. $^{*}p < 0.05$; $^{**}p < 0.01$, *post hoc* comparisons after significant main effects in ANOVAs. The *dashed line* is the forelimb performance recorded in the whole set of rats after 6-OHDA-induced lesions, in a drug free condition before starting the drug treatments.

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the rats during the preceding "agonist stage" of the experiment (Fig. 3G–I).

3.5. Experiment 2: Behavioural effects of D2 agonists when administered de novo

Experiment 1 showed that pramipexole, but not cabergoline, was able to induce AIMs in rats that were rendered dyskinetic by previous exposure to levodopa. To determine whether *de novo* administration of a therapeutically effective dose of pramipexole can induce AIMs, drug-naïve 6-OHDA-lesioned rats were treated with levodopa, cabergoline or pramipexole for 10 days at the same doses used in Experiment 1 (Fig. 4). After 10 days of treatment, cabergoline (2 mg/kg) and pramipexole (1 mg/kg) were equipotent inductors of contralateral turning behaviour when compared with 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation) (Fig. 4A; one-way ANOVA, $p = 0.272$). In contrast, neither cabergoline nor pramipexole improved contralateral forelimb performance in the cylinder test (Fig. 4B; one-way ANOVA $F_{(2,18)} = 8.800$, $p = 0.002$; $^{**}p < 0.01$ versus levodopa) or induced AIMs (Fig. 4C; Kruskal–Wallis ANOVA $H_{(2,21)} = 16.94$, $p = 0.0002$; $^{*}p < 0.05$ versus levodopa). In an attempt to reach a therapeutic effect similar to that of levodopa with D2 agonist mono-therapy, we increased the D2 agonist dosage in a stepwise fashion, and continued treating the rats for 10 additional days (Fig. 4D–I). Cabergoline did not lead to an improvement in the performance of the animals in the cylinder test (Fig. 4E and H), and did not induce AIMs (Fig. 4F and I), at the highest dose tested (4 mg/kg), which was twice the effective dose used in rats previously exposed to levodopa in Experiment 1. In addition, the contralateral turning response was replaced by ipsilateral turning at the higher doses (Fig. 4D and G). In contrast, increasing the dose of pramipexole by 50% (to 1.5 mg/kg) led to an improved performance of the contralateral forelimb in the cylinder test which was not different to the performance elicited by the reference dose of 50 mg/kg of levodopa (corresponding to 250/25 levodopa/carbidopa formulation) (Fig. 4E; one-way ANOVA, $F_{(2,17)} = 12.75$, $p = 0.0004$; $p > 0.05$ pramipexole versus levodopa). This therapeutic benefit was achieved without inducing AIMs (Fig. 4F; Kruskal–Wallis ANOVA: $H_{(2,\ 21)} = 9{,}92$ $p = 0{,}007$; $^{*}p < 0.05$ levodopa versus cabergoline and pramipexole). Increasing further the dose of pramipexole to 2 mg/kg led to a preferential use of the contralateral forelimb in the cylinder test (Fig. 4H; one-way ANOVA, $F_{(2,17)} = 6.47$, $p = 0.008$; $^{*}p < 0.05$, pramipexole or levodopa versus cabergoline) but still did not provoke AIMs (Fig. 4I; Kruskal–Wallis ANOVA: $H_{(2,21)} = 10$, $p = 0.006$, $^{**}p < 0.01$ levodopa versus cabergoline and pramipexole). Three of the rats died during the course of treatment at the highest doses tested (not included here).

To determine whether *de novo* chronic treatment with D2 agonists modified the likelihood of inducing dyskinesias during subsequent chronic levodopa administration, after a 10 day wash out all rats were treated for 10 days with 50 mg/kg levodopa (corresponding to 250/25 levodopa/carbidopa formulation) and examined for AIMs at treatment days 1, 5 and 10 (Fig. 5). After 5 days under levodopa, all rats developed AIMs, and AIMs scores were similar regardless of the drug used in the *de novo* treatments (Kruskal–Wallis ANOVA, $H_{(2,21)} = 1.514$, $p = 0.469$). A few rats showed AIMs at the first levodopa challenge, 3 out of 8 in the *de novo* levodopa group, 1 out of 6 in the *de novo* pramipexole group, and none of 7 in the *de novo* cabergoline group. Note that median AIM scores induced by levodopa were similar to those seen in Experiment 1 (Fig. 3). These results suggest that neither the dopamine receptor selectivity of the *de novo* treatments nor the plasma half-life of the D2 agonists, had a substantial effect on the likelihood of inducing LID in rats with severe lesion to the nigrostriatal pathway.

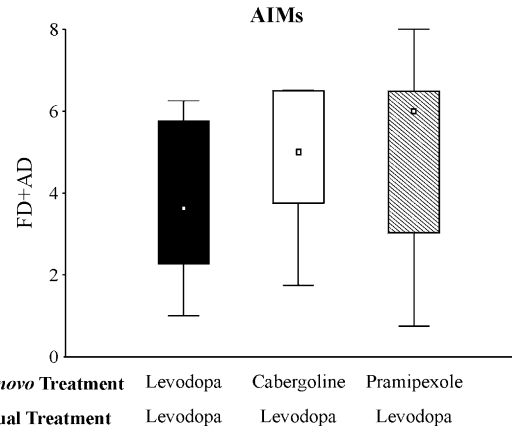

Fig. 5. Effect of *de novo* D2 agonists on levodopa-induced AIMs. AIMs score under levodopa treatment. Regardless of prior treatment, after 5 daily challenges, levodopa induced similar AIM scores in the three rat groups of Experiment 2.

4. Discussion

In rats with extensive nigrostriatal degeneration induced by 6-OHDA we assessed the likelihood of inducing AIMs during chronic treatments with levodopa and D2 agonists, in relationship with: (i) the therapeutic efficacy of the treatments; (ii) prior induction of LID; (iii) *de novo* chronic treatment with D2 agonists. Abnormal involuntary movements were evaluated with a scoring method validated for rodents and therapeutic efficacy by assessing spontaneous purposeful forelimb movements. The main findings of the present study were: (i) prior induction of LID primes 6-OHDA-lesioned rats for the subsequent occurrence of AIMs during mono-therapy with pramipexole (D2 agonist with relatively short half-life) but not with cabergoline (D2 agonist with very long half-life); (ii) once LID has been previously established, an intervening period of mono-therapy with D2 agonists does not modify the severity of AIMs during subsequent mono-therapy with levodopa; (iii) *de novo* treatment with D2 agonists does not modify AIMs during subsequent mono-therapy with levodopa. An important and rather unexpected finding of the present study was that prior chronic levodopa therapy sensitized rats to the therapeutic effects of D2 agonists given in mono-therapy.

In recent years it became evident that rodents with lesions to the nigrostriatal system can display a wide repertoire of motor deficits and abnormal involuntary movements similar to those seen in individuals with PD [6,16,26,37]. However, there is a debate as to which behavioural alterations resemble more closely the clinical features and drug-induced abnormal movements seen in patients. Early studies in the 6-OHDA rat model assumed that contralateral turning behaviour could be predictive of the therapeutic potential of drug treatments [38,39]. However, therapeutic efficacy and potential for the induction of AIMs might be closely interrelated [22,34], and nowadays it seems unlikely that turning behaviour allows for the distinction between both phenomena. Novel tests may allow the evaluation of therapeutic efficacy and AIMs separately. In the cylinder test rats purposefully use their forelimbs to make contact with the cylinder wall and experienced observers can distinguish normal movements from abnormal dyskinetic forelimb movements. Moreover, AIMs of the forelimb and trunk can be scored separately from turning behaviour and normal movement [16]. This view is supported by the present finding that *de novo* administration of D2 agonists can induce strong turning behaviour without improving spontaneous purposeful use of the forelimb or inducing forelimb dyskinesia or axial dystonia. Therefore, in the present study, therapeutically effective doses are

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defined as those reversing forelimb use impairment in the cylinder test.

In rats with established LID, subsequent mono-therapy with pramipexole or cabergoline produced a therapeutic effect similar to that of levodopa despite the severity of the impairment in forelimb use. Importantly, at therapeutically effective doses, levodopa-primed rats exhibited AIMs during mono-therapy with pramipexole but not with cabergoline. The severity of dyskinesias observed in the pramipexole group was somewhat less than in the levodopa group, without reaching statistically significant differences. This is in line with results observed in MPTP lesioned, levodopa-primed, monkeys that showed less severe dyskinesias when switched over to ropinirole [15]. This result extends our previous findings showing that priming 6-OHDA-lesioned rats with the non-selective agonist apomorphine, at doses inducing strong contralateral turning behaviour, enables the appearance of a dyskinetic effect by the D2 selective agonist quinpirole [8]. Surprisingly, in the case of cabergoline, it was possible to dissociate its therapeutic effect from its dyskinetogenic potential, in rats with established LID. In addition, in monkeys rendered dyskinetic by the administration of levodopa, cabergoline, given subsequently in mono-therapy, was able to induce dyskinesias initially, however, after a few weeks dyskinesias waned without the appearance of tolerance to the antiparkinsonian effect [13]. We found that cabergoline did not induce AIMs in rats with established LID, when given in monotherapy. It seems possible that tolerance developed while the plasma concentration of cabergoline was still rising and before the threshold to induce dyskinesia was attained.

Several pharmacological strategies aimed at reversing LID have been tried in patients with little or no success [23,34,35]. In a single observational uncontrolled study, Facca and Sanchez Ramos reported on a limited number of patients that were switched over from levodopa to monotherapy with a dopamine agonist showing no dyskinesias, however, at the expense of significantly reduced motor function [9]. On the contrary, in our first set of experiments, those rats that developed dyskinesias under monotherapy with pramipexole showed comparable improvement in motor function to levodopa. It has been reported that after LID has been established in parkinsonian monkeys, switching to long-acting D2 agonist mono-therapy may "de-prime" the basal ganglia and reduce dyskinesias during subsequent levodopa mono-therapy [13]. We were unable to confirm this finding in the 6-OHDA rat model. After a 10-day wash out aimed at clearing D2 agonists, all rats exhibited similar AIM scores under levodopa regardless of prior treatment with levodopa or D2 agonists by the 5th day. In addition to species differences and other factors that may explain this discrepancy, the 4 day wash out allowed by Hadj Tahar et al [13] may have been insufficient to clear cabergoline from plasma, so reduced LID could be related to more sustained stimulation of D2 receptors by residual cabergoline. This interpretation is consistent with results by Bélanger et al. [1] showing that simultaneous administration of a small dose of cabergoline and a high dose of levodopa could prevent LID.

Several studies concluded that *de novo* administration of D2 agonists in early PD is associated with a low risk of motor complications [5,14,33,34]. In the 6-OHDA rat model a therapeutic effect of pramipexole similar to that of levodopa could be attained without any evidence of AIMs. This was despite pramipexole dose was increased by 50% to be therapeutically effective in non-primed rats. On the other hand, we could not demonstrate a clear therapeutic effect of *de novo* cabergoline at a dose 100% higher than that which was therapeutically effective in levodopa-primed rats. These results support that *de novo* D2 agonist therapy has a very low risk of dyskinesias and extends previous findings by showing that, in addition to priming 6-OHDA-lesioned rats for D2 agonist induced turning behaviour, AIMs and c-*fos* expression [8,20,32], prior non-selective dopamine receptor stimulation enhances or even enables the therapeutic effects of D2 agonists. It seems that, without prior sensitization by repeated levodopa, continuous and progressively higher D2 receptor stimulation induced tolerance to both the therapeutic and dyskinetic D2 agonist effects. This is supported by the fact that *de novo* cabergoline induced strong contralateral turning behaviour initially, but turning rates decreased progressively during chronic treatment and ultimately reversed to ipsilateral turning behaviour at high doses, suggesting the development of significant tolerance to D2 agonist stimulation in the denervated side.

It is believed that *de novo* administration of D2 agonists in early PD delays the appearance of LID [5,14,34]. This delaying effect may be related to a protective anti-dyskinetic effect of *de novo* D2 agonist mono-therapy or just reflect the holdup of levodopa therapy [36]. We found that chronic treatment with D2 agonists did not delay or reduce the intensity of AIMs induced by subsequent mono-therapy with levodopa. This finding is in line with recent retrospective analyses of clinical trials of *de novo* D2 agonist mono-therapy in PD, showing that once levodopa is started, the risk of LID was similar than that of patients treated *de novo* with levodopa [7,36]. Overall, our results do not support that D2 agonist mono-therapy prevents the development of LID or can revert the dysfunction underlying it.

A last issue concerns the different effects of the two D2 agonists used in the present study. In particular, tolerance seemed to appear during cabergoline but not pramipexole administration. The plasma concentration of cabergoline should have increased steadily during our daily treatment because of its very long plasma half-life of about 90 h, while daily periods of low receptor occupancy likely occurred in pramipexole-treated rats. It seems likely that daily periods of very low dopamine receptor occupancy enabled AIM induction and therapeutic efficacy. Remarkably, prior levodopa enabled a therapeutic effect of cabergoline that was not associated with AIMs, suggesting that in addition to sensitizing to dopamine agonist effects (or as part of the mechanism underlying sensitization) priming might disable tolerance to D2 agonist effects. Our findings suggest that tolerance to the therapeutic and dyskinetic effects of continuous D2 receptor stimulation could be disabled separately; raising the hope that therapeutic efficacy might eventually be separated from the dyskinetogenic potential of these drugs.

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