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Short communication

Dopamine reuptake inhibition and failure to evoke dyskinesia in MPTP-treated primates

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Abstract

Nonspecific monoamine reuptake inhibitors reverse motor abnormalities in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets without evoking established dyskinesia. However, it is not known whether dopamine reuptake inhibition alone explains these actions or whether noradrenaline and/or serotonin reuptake blockade also contributes. L-DOPA (12.5 mg/kg, p.o.) rapidly reversed the baseline locomotor deficits and motor disabilities, but evoked dyskinesia (especially limb chorea) in MPTP-treated common marmosets primed to exhibit involuntary movements. In contrast, the selective dopamine reuptake inhibitor 1-(2-(bis-(4-fluorophenyl)-methoxy)ethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (GBR 12909) reversed motor deficits in a dose-dependent manner but, unlike L-DOPA, did not evoke established dyskinesia in these animals. Therefore, inhibition of dopamine reuptake does not evoke established dyskinesia in MPTP-treated primates.

Keywords: GBR 12909; Dyskinesia; Dopamine reuptake inhibition; Parkinson's disease; MPTP-treated primate

1. Introduction

The motor symptoms of Parkinson's disease arise from a reduced striatal dopamine content following nigral cell degeneration (Ehringer and Hornykiewicz, 1960). Dopamine replacement therapy using L-DOPA is the most commonly employed treatment but chronic use frequently induces involuntary movements that can become progressively severe and treatment limiting (Marsden, 1990; Hurtig, 1997; Ahlskog and Muenter, 2001). Dopamine receptor agonists have a lower propensity than L-DOPA to induce dyskinesia when used as early monotherapy but they evoke established involuntary movements in patients previously treated with L-DOPA (Montastruc et al., 1994; Lieberman et al., 1997). Alternative strategies are being sought which are effective in reversing the motor deficits of Parkinson's disease but which will avoid established dyskinesia and monoamine reuptake inhibition may be one such approach.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated common marmosets display most of the motor abnormalities that characterise Parkinson's disease and animals previously primed with L-DOPA exhibit involuntary movements when challenged with a variety of dopaminergic agents (Jenner et al., 1984; Pearce et al., 1995). However, in this model, the potent and nonselective monoamine reuptake inhibitors brasofensine (K_i dopamine/noradrenaline/serotonin uptake inhibition, 3.3/1.3/13 nM; Dr. J. Scheel-Krueger, personal communication) and BTS 74 398 (1-[1-(3,4-dichlororphenyl)cyclobutyl]-2-(3-diaminethylaminopropylthio) ethanone monocitrate; K_i 4.0/7.0/19 nM; Cheetham et al., 1998) are able to reverse motor abnormalities without evoking established dyskinesia (Hansard et al., unpublished observations; Pearce et al., in press). We have previously shown that the ability of the nonselective reuptake blockers to reverse the motor abnormalities is dependent on inhibition of the dopamine transporter (Hansard et al., in press). However, it is not known if dopamine reuptake inhibition also accounts for the lack of dyskinesia observed with brasofensine and BTS 74 398. We now evaluate the ability of GBR 12909 (1-(2-(bis-(4-fluorophenyl)-methoxy)ethyl)-

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4-(3-phenylpropyl) piperazine dihydrochloride), the selective dopamine reuptake inhibitor (Heikkila and Manzino, 1984; K_i dopamine/noradrenaline/serotonin uptake inhibition, 42/624/480 nM; Dr. S.C. Cheetham, personal communication), to reverse the motor abnormalities without evoking dyskinesia in L-DOPA-primed, MPTP-treated common marmosets.

2. Method

2.1. Materials

Drugs were obtained from the following sources: GBR 12909 (1-(2-(bis-(4-fluorophenyl)-methoxy)ethyl)-4-(3-phenylpropyl)piperazine) dihydrochloride (Tocris Cookson, Bristol, UK); L-DOPA (L-3,4-dihydroxyphenylalanine) methyl ester (Sigma, Poole, UK); MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) hydrochloride and carbidopa (Research Biochemicals International, Gillingham, UK).

2.2. Animals

Four adult common marmosets (*Callithrix jacchus*, 348–458 g) were employed. The animals were housed alone or in pairs under standard conditions as detailed earlier (Hansard et al., in press). All procedures were performed in accordance with Home Office regulations (Animals Scientific Procedures Act 1986) under project license number 70/03563.

2.3. Drug administration

Motor abnormalities were induced by MPTP hydrochloride 10.0 mg/kg (subcutaneously dissolved in 0.9% sterile saline) administration over 5 consecutive days as described previously (Hansard et al., in press). The animals were hand-fed for the next 6–8 weeks. Once the animals had overcome the acute effects of MPTP treatment, they were primed for 22–29 consecutive days with once-daily administration of carbidopa (12.5 mg/kg) followed 45–60 min later by L-DOPA methyl ester (10.0–12.5 mg/kg). A recovery period of more than 1 month was allowed before the study was started.

Drugs were administered once a week to allow for washout between treatments. Vehicle, L-DOPA 12.5 mg/kg or GBR 12909 5.0 or 10.0 mg/kg were administered using an open latin square design. GBR 12909 doses were selected on the basis of previous dose–response data (Hansard et al., in press). Carbidopa (12.5 mg/kg, p.o.) pretreatment was given 45–60 min prior to drug administration. Unless otherwise stated, all drugs were orally administered via gavage in 10% sucrose and all dosages refer to the active moiety and not the full or derivative forms.

2.4. Locomotor and behavioural assessment

In brief, the animals were weighed, pretreatment with carbidopa administered, then placed into individual photocell activity cages and left to acclimatise to the test environment for 45–60 min. At this point, vehicle or drug was administered and the animals returned to the activity cages. Locomotor activity, measured as the number of photocell interruptions, was recorded over the following 10 h in 10-min time periods.

Motor disability and dyskinesia exhibited by the animals were graded and scored by a blinded observer as previously described (Pearce et al., 1995; Hansard et al., in press) at the same time as recording locomotor activity. Scores were assessed every 10 min for the first 3 h, over the last 10 min of each 30-min time period for hours 3–6 and over the last 10 min of each hour for hours 6–9. Posture, coordination, balance, alertness, motility, head checking movements and reactions were all assessed. Based on these criteria, a score of zero indicates a normal marmoset and a maximum score of 18 indicates a marmoset with marked motor disability. Similarly a dyskinesia score of zero indicates that no dyskinesia was observed while a maximum score of 4 indicates an animal exhibiting marked and frequent involuntary movements (Pearce et al., 1995). The chorea and dystonia components of dyskinesia were also graded as zero (no chorea or dystonia) and up to a maximum score of 3 (frequent and marked chorea or dystonia; Pearce et al., 1995).

2.5. Statistical analysis

Locomotor activity counts were summed over 30-min time periods and assessed over 4 h. The disability and dyskinesia scores were averaged over 4 h. The Mann–Whitney *U*-Test was used to assess differences between treatment groups with significance set at $P<0.05$. All data are the means ± S.E.M. for four animals.

3. Results

3.1. The effect of L-DOPA on motor abnormalities and the expression of dyskinesia

At baseline, MPTP-treated common marmosets exhibited bradykinesia or akinesia. Animals showed marked motor disability (Fig. 1A) with impaired balance, poor coordination, hunched posture, reduced alertness and an absence of vocalisation. Accordingly, the disability scores were high (Fig. 1B). At baseline, the dyskinesia scores were low and any involuntary movements were infrequent mild, dystonic movements with an absence of chorea (Fig. 1C).

L-DOPA (12.5 mg/kg, p.o.) reversed the locomotor deficits produced by MPTP treatment (Fig. 1A). Bradykinesia and akinesia were rapidly reversed and the effects of

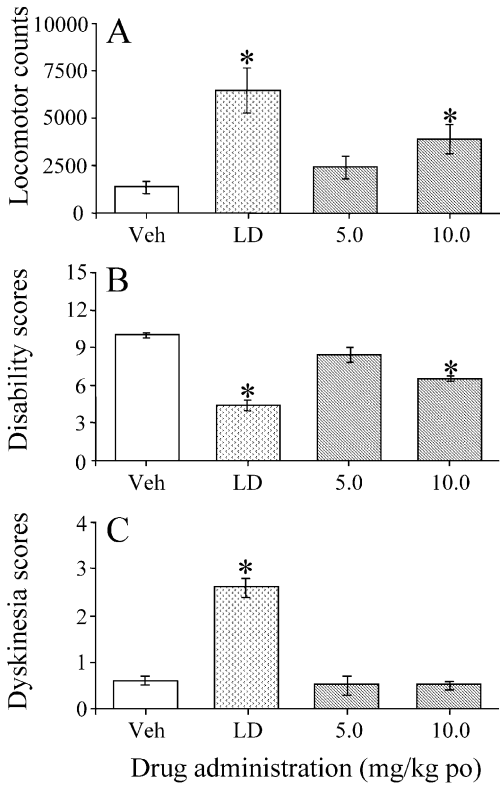

Fig. 1. The activity of GBR 12909 on locomotor activity, motor disability and dyskinesia in L-DOPA-primed, MPTP-treated common marmosets. Locomotor deficits and motor disability were induced in four adult marmosets by the administration of MPTP. Animals were subsequently primed with L-DOPA for 22–29 days in order to establish dyskinesia. Either vehicle (Veh, □), L-DOPA (LD, ▨) 12.5 mg/kg, p.o., or GBR 12909 (▩) 5.0 or 10.0 mg/kg, p.o., were administered. Locomotor counts are the accumulated number of photocell interruptions for 4 h (A). Motor disability was graded (0, normal to 18, severely impaired animal) by a blinded observer averaged over 4 h (B). Dyskinesia was graded (0, absent to 4, severe and frequent) over 4 h (C). All data are the means ± S.E.M. of four animals. * $P < 0.05$ drug versus vehicle.

L-DOPA lasted for up to 4 h (data not shown). Locomotor activity over this time period was significantly enhanced compared to that observed at baseline and appeared driven rather than natural (Fig. 1A). Significant reductions in motor disability were observed which included improvements in alertness, head checking movements, posture, balance and coordination (Fig. 1B). The administration of L-DOPA resulted in marked dyskinesia predominately involving choreic movements of the limbs (Fig. 1C).

3.2. The effect of GBR 12909 on motor abnormalities and dyskinesia expression

GBR 12909 (5.0 mg/kg, p.o.) did not significantly increase locomotor activity, reverse motor disability or evoke established dyskinesia (Fig. 1). In contrast, GBR 12909 10.0 mg/kg significantly increased locomotor activity and reduced motor disability (Fig. 1A and B). A variety of floor, climbing and perch activities were apparent. Balance, coordination, posture, alertness and head checking movements were markedly improved. Administration of GBR 12909 did not result in the expression of dyskinesia over levels observed following vehicle treatment (Fig. 1C). Only infrequent mild dystonic movements were observed. No chorea was apparent.

4. Discussion

The factors responsible for priming basal ganglia for the appearance of dyskinesia are not known. Similarly, the mechanism involved in the expression of dyskinesia are disputed but thought to involve an imbalance between the direct and indirect striatal output pathways (Jenner, 2000; Obeso et al., 2000). Established dyskinesia is provoked by acute challenge with L-DOPA and dopamine receptor agonist drugs but very surprisingly the nonspecific monoamine reuptake inhibitors brasofensine and BTS 74 398 exhibit antiparkinsonian activity without evoking dyskinesia in L-DOPA-primed, MPTP-treated marmosets (Hansard et al., unpublished observations; Pearce et al., in press). Pharmacological analysis has shown that the antiparkinsonian activity of reuptake blockers is due to their ability to inhibit dopamine reuptake and that additional noradrenergic and serotoninergic reuptake blocking activity may inhibit this effect (Hansard et al., in press). However, it is not known if the failure to induce dyskinesia is also related to dopamine reuptake blockade or whether this is a feature of the nonspecific action of the monoamine reuptake blockers studied. This study is the first report of a selective dopamine reuptake inhibitor administered to MPTP-treated primates in a model of dyskinesia.

At the highest dose used, the selective dopamine reuptake inhibitor GBR 12909 reversed the locomotor deficits produced by MPTP treatment in a manner comparable to the effect produced following L-DOPA administration. However, GBR 12909 did not evoke the dyskinetic response demonstrated by L-DOPA. The antiparkinsonian potential of GBR 12909 is in agreement with previous studies demonstrating that dopamine reuptake blockade is important for the antiparkinsonian potential of monoamine reuptake inhibitors (Hansard et al., in press). The lack of dyskinesia produced by GBR 12909 as well as the nonselective monoamine reuptake inhibitors (Hansard et al., unpublished observations; Pearce et al., in press) suggests that dopamine reuptake blockade is the key to why these agents do not provoke involuntary movements.

Why GBR 12909 and other monoamine reuptake inhibitors do not induce established dyskinesia requires explanation, since presumably dopamine reuptake inhibition would produce activation of striatal dopamine receptors in the same manner as L-DOPA or dopamine receptor agonists. Activation of dopamine D_1 receptors is associated with less established dyskinesia in MPTP-treated primates than with dopamine D_2 receptor stimulation (Gomez-Mancilla and

Bedard, 1991; Pearce et al., 1999; Jenner, 2000). Interestingly, inhibition of dopamine reuptake may preferentially activate dopamine D_1 receptors and this might explain the lack of dyskinesia (Rosenzweig-Lipson et al., 1994; Xu et al., 1994; Pearce, 1996).

Alternatively, there may be differences between the brain regions in which monoamine reuptake inhibitors and L-DOPA produce their effects. L-DOPA presumably acts predominately on the dopamine denervated striatum to produce its effect whereas monoamine reuptake inhibitors may act preferentially in the mesolimbic pathway which is largely unaffected by MPTP treatment (Burns et al., 1983; Langston et al., 1984; Chiueh et al., 1985; Cass et al., 1993 and references cited therein). Studies of Fos-immunoreactivity are underway in 6-hydroxydopamine lesioned rats to determine if BTS 74 398 preferentially acts on the nucleus accumbens whereas L-DOPA predominately affects the dorsal striatum.

In conclusion, this study suggests that selective dopamine reuptake inhibition has antiparkinsonian activity without evoking established dyskinesia in L-DOPA-primed, MPTP-treated common marmosets. Dopamine reuptake blockers may provide a novel means of treating Parkinson's disease as, unlike other current therapies, they can dissociate antiparkinsonian activity from involuntary movements.

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