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Short communication

Antidyskinetic effect of JL-18, a clozapine analog, in parkinsonian monkeys

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Abstract

Clozapine reduces L-3,4-dihydroxyphenylalanine (L-Dopa)-induced dyskinesias in parkinsonian patients. To test if the antidyskinetic effect of clozapine is related to antagonism at the dopamine D_4 receptor, we investigated the effect of 8-methyl-6-(4-methyl-1-piperazinyl)-11H-pyrido[2,3-b][1,4]benzodiazepine (JL-18), a structural analog of clozapine which is more selective for this receptor. Four 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated cynomolgus monkeys with a stable parkinsonian syndrome and reproducible dyskinesias to L-Dopa were used in this study. They were injected subcutaneously (s.c.) with L-Dopa methyl ester (125 mg per animal) plus benserazide (50 mg per animal; L-Dopa/benserazide) alone or in combination with JL-18 (at the doses of 0.1, 0.3, or 0.9 mg/kg, s.c.). Subcutaneous injection of sterile saline was used as control. L-Dopa/benserazide increased locomotion and improved parkinsonism but also induced dyskinesias. Co-administration of JL-18, at low doses (0.1, 0.3 mg/kg) with L-Dopa/benserazide, produced a dose-dependent reduction in L-Dopa-induced dyskinesias without a parallel return to parkinsonism. The present results suggest that novel selective dopamine D_4 receptor antagonists may represent a useful tool to reduce L-Dopa-induced dyskinesias.

Keywords: Dopamine D_4 receptor; L-Dopa-induced dyskinesia; JL-18; Parkinson's disease; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-Dopa)-induced dyskinesias, the most common side effect of current antiparkinsonian treatments, are extremely difficult to control and their pathophysiology is poorly understood (Crossman, 1990; Marsden, 1994). Both clinical observations and experimental studies of L-Dopa-induced dyskinesias suggest that exogenous exposure to dopaminergic drugs, mainly when given intermittently for a long time, and a severe dopamine denervation are crucial factors (Chase, 1998).

Dopamine neurotransmission is mediated by at least five receptors differentiated into two major subfamilies, D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4 and their variants) (Missale et al., 1998). Dopamine receptor supersensitivity is thought to be one of the major contributing factors for the development of L-Dopa-induced dyskinesias, and reports from the literature include both dopamine D_1 and D_2 receptor-mediated events. Dopamine D_1 receptor-mediated mechanisms have traditionally been linked primarily to this phenomenon (Blanchet et al., 1994). However, Grondin et al. (1999), using dopamine D_1 receptor selective antagonists have shown that D_1 receptor blockade improved L-Dopa-induced dyskinesias but also worsened parkinsonism in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. On the other hand, dopamine D_2 receptor mechanisms have also been implicated in L-Dopa-induced dyskinesias. Again, the adjuction of raclopride, a classical dopamine D_2 receptor antagonist to L-Dopa reduced L-Dopa-induced dyskinesias but with a return to akinesia in MPTP-lesioned common marmosets (Ekesbo et al., 1997). Similarly, other dopamine D_2 receptor antagonists, which impede dopaminergic transmission, improve dyskinesias at the cost of a return to parkinsonism (Grondin et al., 1999; Klawans and Weiner, 1974).

In contrast with dopamine D_1 and D_2 receptors, little is known about the implication of dopamine D_3, D_4 and D_5

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receptors in L-Dopa-induced dyskinesias. Interestingly, the atypical neuroleptic clozapine reduces L-Dopa-induced dyskinesias in Parkinson's disease patients when added to current therapy, without impairing the relief of symptoms (Durif et al., 1997; Bennett et al., 1994 ; Schuh and Bennett, 1993). Moreover, in MPTP-treated monkeys, co-administration of the lower doses of clozapine decreased significantly L-Dopa-induced dyskinesias without impairing the relief of parkinsonism or the L-Dopa/benserazide-increase in locomotion (Grondin et al., 1999). Clozapine has potent antidopaminergic, but also antimuscarinic, antiadrenergic and antiserotoninergic effects (Brunello et al., 1995; Aschby and Wang, 1996). At the dopamine receptors, clozapine has higher affinity for dopamine D_4 over D_1, D_2, and D_3 receptors, and this seems to contribute to its antipsychotic action (Brunello et al., 1995).

To investigate if the antidyskinetic effect of clozapine is related to dopamine D_4 antagonism, we have studied the effects of 8-methyl-6-(4-methyl-1-piperazinyl)-11H-pyrido[2,3-b][1,4]benzodiazepine (JL-18), a pyridobenzodiazepine derivative bioisoster of clozapine, which is more selective at dopamine D_4 receptors (Liegeois et al., 1995), on the antiparkinsonian response and dyskinesias produced by L-Dopa/benserazide in MPTP-treated cynomolgus monkeys.

2. Materials and methods

2.1. Animals

Four adult female cynomolgus (*Macaca fascicularis*) monkeys weighing 3.3–4.3 kg were used in this study in accordance with the standards of the Canadian Council on Animal Care. All animals had a stable parkinsonian syndrome induced by weekly injections of MPTP and reproducible dyskinesias to L-Dopa (as described in Grondin et al., 1999). Prior to this study, all animals have been already treated with L-dopa or dopamine receptor agonists (such as dopamine D_1 receptor agonist: 6-chloro-7,8-dihyroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazeine hydrobromide (SKF-82958), or dopamine D_2 receptor agonist: quinpirole). However, no other treatment than L-Dopa was given in the month preceding the present study to maintain priming.

2.2. Drugs

L-Dopa methyl ester (L-Dopa) (125 mg per animal; Sigma, St-Louis, Missouri) was dissolved in 0.9% sterile saline (1 ml) and administered subcutaneously (s.c.) always together with benserazide (50 mg, s.c.; Hoffmann–La Roche, Montréal, Québec; L-Dopa/benserazide 125/50 mg). JL-18 (Sigma) was dissolved in sterile water and administered s.c. at the following doses: 0.1, 0.3 or 0.9 mg/kg in combination with L-Dopa/benserazide at the same doses as indicated above. JL-18 was also administered alone at the dose of 0.1 mg/kg. Subcutaneous injection of sterile saline was used as control.

2.3. Evaluation of the response

The *parkinsonian syndrome* following MPTP exposure and the relief of parkinsonism following the administration of L-Dopa/benserazide alone or in combination with JL-18 were rated according to a disability scale for monkeys which we have used in several published studies (Grondin et al., 1999). The *severity of dyskinesias* was rated every 30 min for the face, neck, trunk, arms and legs in the following way: none = 0; mild (occasional) = 1; moderate (intermittent) = 2; severe (continuous) = 3. The dyskinetic score obtained was the sum of the scores for all body segments for a maximal score of 21 points. The score reflected the intensity and frequency of dyskinesias in the preceding 30 min. *Locomotor activity* of all monkeys was monitored using an electronic motility monitoring system fixed on each cage (Datascience, St. Paul, Minnesota). Signals were accumulated by a computer that provided a mobility count every 5 min.

2.4. Statistical analysis

The total mobility counts recorded individually during a 4-h period following a given treatment were averaged for all monkeys and compared using an analysis of variance (ANOVA) for repeated measures followed by a Fisher's PLSD test (least significant differences). The mean parkinsonian scores and the mean dyskinetic scores obtained during each observation interval were averaged for all monkeys. These averaged scores were compared using a nonparametric Friedman's test.

3. Results

L-Dopa/benserazide produced an improvement in motor activity as indicated by the mobility counts which increased significantly as compared to the baseline level (saline treatment, Fig. 1). In contrast, JL-18 administered alone at 0.1 mg/kg had no statistically significant effect on motor activity, as compared to the baseline value (Fig. 1). Co-administration of L-Dopa/benserazide with JL-18, at the low doses tested, produced a significant stimulatory effect on locomotion. However, JL-18, at 0.9 mg/kg, decreased the L-Dopa increase in locomotor activity to the control level (Fig. 1).

Fig. 1 also illustrates the results of the antiparkinsonian and the dyskinetic responses for each drug treatment. The MPTP-treated primates used in this study presented a stable parkinsonian syndrome with an average baseline score of 12.3/16. Administration of L-Dopa/benserazide

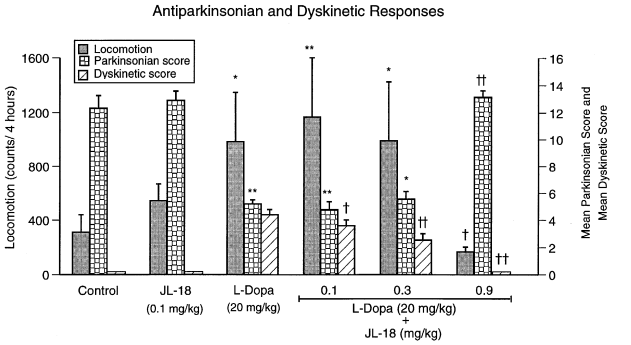

Fig. 1. Locomotion, antiparkinsonian and dyskinetic response after acute treatment with a given drug. The total mobility counts recorded individually during 4 h following a given treatment were averaged for all monkeys and compared using ANOVA for repeated measures followed by a Fisher's PLSD test. The mean parkinsonian scores and the mean dyskinetic scores, respectively, obtained every 30 min during an observed effect were averaged for all monkeys. These averaged scores were compared using the nonparametric Friedman's test. In the case of no behavioral effect was seen (as for saline injections), the scores obtained during a comparable interval of time were considered. Counts ± S.E.M. ($^{*}P < 0.05$ and $^{**}P < 0.01$ vs. saline; $^{\dagger}P < 0.05$ vs. L-Dopa/benserazide). Parkinsonian scores ± S.E.M. ($^{*}P < 0.05$ and $^{**}P < 0.01$ vs. saline; $^{\dagger\dagger}P < 0.01$ vs. L-Dopa/benserazide). Dyskinetic scores ± S.E.M. ($^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ vs. L-Dopa/benserazide).

improved the disability features and lowered the parkinsonian score significantly as compared to the baseline (control) score (Fig. 1). On the other hand, s.c. injection of JL-18 (alone at 0.1 mg/kg) had no significant effect on parkinsonism (Fig. 1). Co-administration of JL-18 with L-Dopa/benserazide at low doses did not worsen parkinsonism and there was a clear antiparkinsonian effect when compared to baseline (Fig. 1). However, JL-18 at 0.9 mg/kg completely counteracted the antiparkinsonian effect of L-Dopa/benserazide.

L-Dopa/benserazide administered alone induced dyskinesias. These dyskinetic movements were moderate in intensity and choreic and/or dystonic in nature. No dyskinesias were observed after s.c. administration of JL-18 alone at a dose of 0.1 mg/kg (Fig. 1). The co-administration of JL-18 with L-Dopa/benserazide was followed by a dose-dependent decrease in dyskinesias (mean dyskinetic scores; Fig. 1). The inhibition of dyskinesias with the lower doses of JL-18 was not related to sedation as locomotor activity levels were still high when compared to baseline (Fig. 1).

On the other hand, the behavioral response to L-Dopa/benserazide given alone occurred 9.4 ± 0.5 min after s.c. administration and lasted 144.6 ± 5.9 min (data not shown). Co-administration of JL-18 at low doses with L-Dopa/benserazide had a small effect on the delay for the onset of the anti-parkinsonian response (a slight increase by 7 min). Thus, the total duration of L-Dopa effects was not affected (slightly decreased by 10 min). However, the behavioral effect was abolished at the higher dose of JL-18 tested.

4. Discussion

Grondin et al. (1999) have recently shown that co-administration of low doses of clozapine decreased significantly L-Dopa-induced dyskinesias without impairing the L-Dopa improvement of parkinsonian score and locomotor activity in the same model of Parkinson's disease. Clozapine has antagonistic actions not only on dopamine D_4, but also on D_1 receptors. However, the selective dopamine D_1 receptor antagonists, (+)-8-Chloro-3-methyl-5-(7-benzofuranyl)-2,3,4,5-tetrahydro-1H-3-benzazepine-7-ol (NNC 01-112) and *R*-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydroxy-1H-3-benzazepine maleate (SCH 23390), improve L-Dopa-induced dyskinesias but worsen parkinsonism in MPTP monkeys (Grondin et al., 1999). The clozapine-like antidyskinetic effect of JL-18, which presents lower affinity for dopamine D_1 receptor but more selective for dopamine D_4 receptors (compared to clozapine), suggests a primary role for D_4 receptors in dyskinesias. In fact, JL-18 is a weak dopamine D_2 receptor antagonist and presents a 25-fold greater selectivity for dopamine D_4 receptors than for dopamine D_2 receptors (Liegeois et al., 1995). This is higher than the twofold selectivity of clozapine (Lahti et al., 1993). It should be noted that a 10-fold selectivity of clozapine was also reported (Van Tol et al., 1991). Moreover, JL-18 presents lower affinities, compared to clozapine, for other binding sites — muscarinic acetylcholine and serotonin 5-HT_2 receptor (Liegeois et al., 1995). This also suggests that the antidyskinetic effect of JL-18 observed in this study is more likely to be related to dopamine D_4 antagonism.

Dopamine D_4 receptors may be distributed more widely than was previously thought based on mRNA localisation (Matsumoto et al., 1996). Indeed, recent immunocytochemistry studies showed the presence of dopamine D_4 receptors both in limbic and motor areas of the rat, primate and human brain (Defagot et al., 1997; Khan et al., 1998; Mrzljak et al., 1996). Interestingly, dopamine D_4 receptor immunoreactivity was detected in neurons of both segments of the globus pallidus and the pars reticulata of the sustantia nigra (Mrzljak et al., 1996), which are known to release the inhibitory neurotansmitter γ-aminobutyric acid (GABA). Thus, by its localization on GABA(ergic) neurons of the output nuclei of basal ganglia thedopamine, D_4 receptor could modulate the GABA(ergic) transmission in these nuclei. In fact, an excessive decrease in the activity of the basal ganglia output nuclei have commonly been suggested implicated in the pathophysiology of L-Dopa-induced dyskinesias (Crossman, 1990).

Recently, in an autoradiographic study, Tarazi et al. (1998) reported the presence of D_4-like receptor in the striatum both on intrinsic postsynaptic neurons and on presynaptic corticostriatal afferents (Tarazi et al., 1998; Tarazi and Baldessarini, 1999). Dopamine D_4 receptors on corticostriatal afferents might modulate glutamatergic neurotransmission in the striatum, as well as in cerebral cortex

(Tarazi et al., 1998). In fact, glutamate release and electrophysiological studies, using non-selective D_2-like compounds, have associated inhibitory effects of dopamine on glutamatergic neurons with D_2 receptors (Tarazi et al., 1998; Yamamoto and Davy, 1992). Interestingly, recent observations show that dysfunction of some glutamatergic systems in the basal ganglia can be related to the development of L-Dopa-induced dyskinesias. For instance, systemic administration of an NMDA antagonist to MPTP-treated monkeys causes suppression of L-Dopa-induced dyskinesias while leaving the antiparkinsonian response to L-Dopa unaltered (Papa and Chase, 1996). Moreover, improvement of L-Dopa-induced dyskinesias with NMDA antagonists was also reported in parkinsonian patients (Verhagen Metman et al., 1998). Thus, the antidyskinetic effect of JL-18 may include effects mediated by dopamine D_4 receptors in the striatum and/or in the cortex. Further studies employing emerging agents that are even more selective for dopamine D_4 receptor may help to clarify these predictions.

In conclusion, the present results indicate that JL-18, a structural analog of clozapine which is more selective at dopamine D_4 receptor, has clozapine-like antidyskinetic effect in MPTP monkeys. Thus, novel selective dopamine D_4 receptor antagonists may represent a useful tool to reduce L-Dopa-induced dyskinesias and need further investigations in animal models of Parkinson's disease.

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