Behavioral Involvement of Central Dopamine D₁ and D₂ Receptors in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Lesioned Parkinsonian Cynomolgus Monkeys

Tetsuo Akai¹, Masaki Ozawa¹, Motonori Yamaguchi¹, Eiji Mizuta² and Sadako Kuno²*

¹Research Department, Institute of Pharma Research, Development and Medical Science, Nihon Schering K.K., 2-6-64 Nishimiyahara, Osaka 532, Japan
²Department of Neurology, Center for Neurological Diseases, Utano National Hospital, Narutaki, Kyoto 616, Japan

Received September 14, 1994 Accepted November 10, 1994

ABSTRACT—To clarify the roles of dopamine D₁ and D₂ receptors in behavioral symptoms of Parkinson’s disease, antiparkinsonian effects of various dopamine agonists in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian monkeys were investigated with regard to induction of hyperactivity such as excitability, irritability and aggressiveness. The non-selective dopamine agonist apomorphine ameliorated the parkinsonism, but induced marked hyperactivity dose-dependently. Pretreatment with either the dopamine D₁ antagonist SCH 23390 or the dopamine D₂ antagonist sulpiride markedly suppressed the apomorphine-induced hyperactivity with slight attenuation of the antiparkinsonian effects. Both the dopamine D₂-receptor agonist quinpirole and the dopamine D₁-receptor agonist SKF 82958 ameliorated the parkinsonism in a dose-dependent manner with a slight induction of hyperactivity. Combination treatment of a threshold dose of quinpirole with that of SKF 82958 augmented the antiparkinsonian effects without a marked induction of hyperactivity. However, the combination treatment at higher doses induced marked hyperactivity accompanied by augmented antiparkinsonian effects. These results suggest that stimulation of either central dopamine D₁ or D₂ receptors is requisite for the antiparkinsonian effects and concurrent strong stimulation of both central dopamine D₁ and D₂ receptors causes marked hyperactivity which may be predictive of dopaminergic psychiatric side effects.

Keywords: Parkinsonism, Dopamine D₁ receptor, Dopamine D₂ receptor, MPTP, Monkey

Central dopamine (DA) receptors may be classified into two subtypes (D₁ and D₂) on the basis of structural homology, biochemical properties and pharmacological profiles, although five subtypes of dopamine receptors have recently been identified in various dopaminergic systems of mammalian brains (1, 2). Steadily accumulating evidence indicates that under conditions, concurrent stimulation of both dopamine D₁ and D₂ receptors is required for the manifestation of behavioral or electrophysiological effects in rodents (3), although opposite effects of dopamine D₁ and D₂ agonists on some of oral movements is observed in rats (4). However, the specific roles of these receptors and their interaction in the pathophysiology of Parkinson’s disease remains uncharacterized.

To date, various direct dopamine D₂-receptor agonists such as bromocriptine and lisuride have been evaluated in clinical trials (5, 6). These clinical trials demonstrated that monotherapy with the dopamine D₂-receptor agonist is effective but insufficient to produce an adequate therapeutic response in patients with Parkinson’s disease compared to levodopa (L-DOPA) therapy, suggesting that stimulation of dopamine D₂ receptors is essential, but dopamine D₁ receptors also may play a pivotal role in the treatment of Parkinson’s disease. However, the role of dopamine D₁ receptors has not been clearly determined.

In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys, the partial dopamine D₁-receptor agonist SKF 38393 failed to ameliorate the parkinsonism (7) and suppressed the antiparkinsonian effects of the dopamine D₂-agonist quinpirole (8). On the other hand, the another partial dopamine D₁-receptor agonist CY 208-243 ameliorated the parkinsonism and potentiated the antiparkinsonian effect elicited by bromocriptine (9, 10). Recently full dopamine D₁-receptor agonists such as dihydrexin-
dine and SKF 81297 have been reported to show an anti-
parkinsonian effect (11, 12).

L-DOPA, the direct precursor of dopamine, is still the
most effective drug for the treatment of motor dysfunc-
tion in Parkinson's disease, but has been noticed to in-
duce psychiatric side effects, including delusion, hallucina-
tion and organic confusional states, and motor complica-
tions such as daily fluctuations in motor capability and
dyskinesia (13). Although the cause of these psychiatric
side effects in the L-DOPA therapy remains insufficiently
characterized, the concurrent stimulation of dopamine D1-
and D2-receptor subtypes is believed to result in some of
these side effects.

The present study was performed to clarify the behav-
ioral roles and interactions between dopamine D1 and D2
receptors in Parkinson's disease. We investigated the
behavioral effects of various dopamine agonists, the non-
selective dopamine agonist apomorphine, the dopamine
D1-receptor agonist SKF 82958 (14) and the dopamine D2-
receptor agonist quinpirole in MPTP-lesioned parkinsoni-
an cynomolgus monkeys, with regard to the induction of
hyperactivity such as excitability, irritability and aggres-
siveness.

MATERIALS AND METHODS

Animal preparation

In six cynomolgus monkeys (Macaca fascicularis; Clea
Japan, Inc., Tokyo), three intravenous injections of 0.3
mg/kg of MPTP hydrochloride (Research Biochemicals,
Inc. (RBI), Natick, MA, USA) were given at intervals of
3–4 days. This treatment was followed by several injec-
tions of the same dosage every 7 days. The animals
showed persistent parkinsonian symptoms when the total
cumulative dose reached 1.5 to 2.1 mg/kg. In the experi-
ment with apomorphine treatment, four animals weigh-
ing 3.3 to 4.8 kg with persistent parkinsonism were used
about 1 year after the latest MPTP injection. After that
experiment, one animal died and two animals were added
for the other experiments (finally, five animals weighing
3.3 to 5.2 kg).

Behavioral assessment

Parkinsonism and hyperactivity (irritability, excitability
and aggressiveness) were scored by the rating scale
illustrated in Table 1 according to Clarke et al. (15) with a
slight modification. Behavioral assessments with this scor-
ing scale were carried out by an examiner (T.A.) who was un-
aware of to the treatment conditions. A washout period

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinsonism</td>
<td></td>
</tr>
<tr>
<td>Alertness</td>
<td>normal: 0, reduced: 1, absent: 2</td>
</tr>
<tr>
<td>Head</td>
<td>present: 0, reduced: 1, absent: 2</td>
</tr>
<tr>
<td>checking movement</td>
<td></td>
</tr>
<tr>
<td>Eyes</td>
<td>normal: 0, reduced: 1, eye closed: 2</td>
</tr>
<tr>
<td>attention, blinking, movement</td>
<td></td>
</tr>
<tr>
<td>Posture</td>
<td>normal: 0, abnormal—trunk: +1, limb: +1, grossly abnormal: 3</td>
</tr>
<tr>
<td>Balance</td>
<td>normal: 0, impaired: 1, no movement: 2</td>
</tr>
<tr>
<td>Motility</td>
<td></td>
</tr>
<tr>
<td>at rest</td>
<td>normal: 0, mildly slowing: 1, moderate bradykinesia: 2, bradykinesia: 3, akinesia: 4</td>
</tr>
<tr>
<td>reaction to external stimuli</td>
<td>normal: 0, reduced: 1, slow: 2, absent: 3</td>
</tr>
<tr>
<td>Vocalization</td>
<td>normal: 0, absent: 1</td>
</tr>
<tr>
<td>Tremor</td>
<td>absent: 0, moderate: 1, severe: 2</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td></td>
</tr>
<tr>
<td>Excitability</td>
<td>none: 0, moderate: 1, severe: 2</td>
</tr>
<tr>
<td>chattering, mounting, jumping around</td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>none: 0, moderate: 1, severe: 2</td>
</tr>
<tr>
<td>rotating, jumping, and looking around</td>
<td></td>
</tr>
<tr>
<td>restlessly when threatened</td>
<td></td>
</tr>
<tr>
<td>Aggressiveness</td>
<td>none: 0, moderate: 1, severe: 2</td>
</tr>
<tr>
<td>head lowering, lounging forward, swatting,</td>
<td></td>
</tr>
<tr>
<td>baring teeth, biting displayed toward object</td>
<td></td>
</tr>
</tbody>
</table>
of at least 3 days elapsed between drug treatments.

**Drug administration**

Apomorphine hydrochloride (Sigma, St. Louis, MO, USA) was dissolved into saline solution containing 0.05% ascorbic acid. Quinpirole hydrochloride (RBI), SKF 82958 (RBI) and SCH 23390 (RBI) were dissolved into saline solution. SCH 23390 and sulpiride (Dogmatyl Injection; Fujisawa Pharmaceuticals Co., Ltd., Osaka) were administered 15 and 90 min, respectively, prior to the injection of apomorphine. In the combination study with quinpirole and SKF 82958, quinpirole were administered 15 min prior to the injection of SKF 82958. All drugs were administered subcutaneously with an injection volume of 0.1 ml/kg, except for sulpiride which was administered in an appropriate volume at the concentration of 50 mg/ml.

**Statistics**

Statistical differences were determined by two-way repeated measures analysis of variance (ANOVA) and followed by Dunnett’s tests for multiple comparisons where appropriate. A probability within 0.05 was considered to indicate a significant difference.

**RESULTS**

The non-selective dopamine-receptor agonist apomorphine (0.01 – 0.3 mg/kg, s.c.) ameliorated dose-dependently the symptoms of parkinsonism such as tremor and bradykinesia, and it simultaneously induced marked hyperactivity such as excitability, irritability and aggressiveness (Fig. 1). The hyperactivity disappeared when the parkinsonism was re-established within 2 hr (data not shown).

The selective dopamine D2-receptor antagonist sulpiride (100 mg/kg, s.c.) alone deteriorated the parkinsonism as demonstrated by bradykinesia with transient eyelid closure (data not shown). Pretreatment with sulpiride (10–100 mg/kg, s.c.) markedly suppressed the hyperactivity induced by apomorphine (0.3 mg/kg, s.c.), but induced only slight attenuation of the antiparkinsonian effects of apomorphine (Fig. 2). The selective dopamine D1-receptor antagonist SCH 23390 (0.03 mg/kg, s.c.) alone tended to deteriorate the parkinsonism with transient eyelid closure (data not shown). Pretreatment with SCH 23390 (0.003 – 0.03 mg/kg, s.c.), as did sulpiride, suppressed the hyperactivity. Only the highest dose of SCH 23390 (0.03 mg/kg, s.c.) slightly attenuated the antiparkinsonian effects of apomorphine 0.3 mg/kg, s.c. (Fig. 3). Lower doses of SCH 23390 (0.003 and 0.01 mg/kg, s.c.) did not affect the antiparkinsonian effect of apomorphine.

Both the selective full dopamine D2-receptor agonist quinpirole (0.03 – 0.3 mg/kg, s.c.) and the selective full dopamine D1-receptor agonist SKF 82958 (0.1 – 0.5 mg/kg, s.c.) ameliorated the symptoms of parkinsonism such as tremor and bradykinesia dose-dependently with a slight induction of hyperactivity. The antiparkinsonian effects of quinpirole at 0.3 mg/kg, s.c. lasted more than 3 hr (Fig. 4). The antiparkinsonian effects of SKF 82958 at 0.5 mg/kg, s.c. disappeared within 1.5 hr (Fig. 5).

Combination treatment with threshold doses of quinpirole (0.01 mg/kg, s.c.) and that of SKF 82958 (0.1 mg/kg, s.c.) augmented the antiparkinsonian effects without induction of any hyperactivity. The augmented antiparkinsonian effect by combined treatment with quinpirole and SKF 82958 disappeared within 1 hr after the administration of SKF 82958 (Fig. 6). The combination treatment with higher doses of quinpirole (0.1 mg/kg, s.c.) and SKF 82958 (0.25 mg/kg, s.c.), at which they showed potent antiparkinsonian effects by themselves,
Fig. 2. Effect of sulpiride on behavioral changes induced by apomorphine (Apo.) in MPTP-lesioned monkeys. Sulpiride was administered subcutaneously 90 min prior to the apomorphine injection (0.3 mg/kg, s.c.). Parkinsonism and hyperactivity were scored and accumulated for 120 min after apomorphine injection. The mean values were obtained from 4 monkeys. All values are expressed as a percent ± S.E. of the mean values obtained in the group treated with apomorphine alone. *P < 0.05, **P < 0.01 vs group treated with apomorphine alone.

Fig. 3. Effect of SCH 23390 on behavioral changes induced by apomorphine (Apo.) in MPTP-lesioned monkeys. SCH 23390 was administered subcutaneously 15 min prior to the apomorphine injection (0.3 mg/kg, s.c.). Parkinsonism and hyperactivity were scored and accumulated for 120 min after apomorphine injection. The mean values were obtained from 4 monkeys. All values are expressed as a percent ± S.E. of the mean values obtained in the group treated with apomorphine alone. *P < 0.05, **P < 0.01 vs group treated with apomorphine alone.
Parkinsonism and hyperactivity were scored, and the mean values were obtained from 5 monkeys. Quinpirole: 0.3 (○), 0.1 (△), 0.03 (□) mg/kg, s.c. and saline (◇). *P<0.05, **P<0.01 vs saline treatment group at the same time point after administration.

induced marked hyperactivity transiently and augmented the antiparkinsonian effects. The hyperactivity induced by the combination treatment disappeared within 30 min. The time course of the appearance of the hyperactivity was almost identical to that of the antiparkinsonian effects by SKF 82958 (Fig. 7).

DISCUSSION

In the present study, both the selective dopamine D₁- and D₂-receptor antagonists at the doses tested failed to suppress the antiparkinsonian effects by the non-selective dopamine D₁/D₂-receptor agonist apomorphine. Furthermore, the selective dopamine D₁-receptor agonist SKF 82958 with full agonistic activity as well as the dopamine D₂-receptor agonist quinpirole showed the antiparkinsonian effects. These results are consistent with the previous reports that the selective dopamine D₁-receptor agonists with full agonistic activity such as dihydrexidine and SKF 81297 ameliorate the parkinsonism in MPTP-lesioned monkeys (11, 12). These findings together with the present results indicate that a sufficient stimulation of dopamine D₁-receptors, as well as that of dopamine D₂ receptors, ameliorates the symptoms of Parkinson's disease.

In contrast to apomorphine inducing the marked hyperactivity, either of the selective dopamine D₁- or D₂-receptor agonists alone did not induce marked hyperactivity with further increase in dose. Furthermore, both the selective dopamine D₁- and the D₂-receptor antagonists at the doses tested suppressed the induction of hyperactivity by apomorphine without marked attenuation of the antiparkinsonian effect. In addition, the combination treat-
Fig. 6. Behavioral effects of quinpirole (0.01 mg/kg, s.c.) in combination with SKF 82958 (0.1 mg/kg, s.c.) in MPTP-lesioned monkeys. Quinpirole was administered 15 min prior to SKF 82958 injection. Parkinsonism and hyperactivity were scored, and the mean values were obtained from 5 monkeys. Quinpirole in combination with SKF 82958 (○), quinpirole alone (△), SKF 82958 alone (□) or vehicle (△). *P<0.05, **P<0.01 vs saline treatment group at the same time point after administration.

The interaction patterns of dopamine D₁ and D₂ receptor stimulations were different between the antiparkinsonian effects and the induction of hyperactivity, although both actions were augmented by the combination treatment of the dopamine D₁-receptor agonist with the dopamine D₂-receptor agonist. Antiparkinsonian effects were elicited by either dopamine D₁ or D₂ receptor stimulation. On the other hand, the induction of hyperactivity required simultaneous stimulation of both dopamine D₁ and D₂ receptors. In MPTP-lesioned parkinsonian monkeys, the nigrostriatal dopamine neurons are selectively degenerated, which results in the dopamine depletion and the development of postsynaptic supersensitivity of dopamine receptors in the striatum, whereas MPTP causes less severe degeneration of the mesolimbic and cortical dopamine neurons (16). Under normal conditions, concurrent stimulation of dopamine D₁ and D₂ receptors has been reported to be required for the manifestation of some behavioral or electrophysiological effects (3). However, when the nigrostriatal pathway is disrupted, the actions of dopamine D₁ and D₂ receptors seem to become independent (17). Thus, in MPTP-lesioned parkinsonian monkeys, it can be postulated that some behaviors via mesolimbic/cortical dopamine neurons require...
concurrent stimulation of dopamine D_1 and D_2 receptors, whereas those via nigrostriatal dopamine neurons can be elicited by independent stimulation of either dopamine D_1 or D_2 receptors.

Admittedly, animal studies do not dispose of reliable models of psychotic disorders. Pharmacogenic psychoses induced in the animal are evaluated on the basis of complex and bizarre psychomotor symptoms, which may or may not reflect psychomotor abnormality manifested in humans (18). It should be noted, however, that, in contrast to psychosis in schizophrenia, human pharmacogenic psychoses are induced by a particular group of drugs with a common mechanism of action (e.g., dopamine receptor activation) and confined to a particular subpopulation of neurological disorders (e.g., advanced Parkinson’s disease). Thus, pharmacogenic psychoses are more likely to be induced in an animal model, particularly if the model also mimics the pathophysiology of the underlying human disease as it does in the MPTP-induced parkinsonism in monkeys. If one hypothesizes that in MPTP-lesioned monkeys the dopamine agonist-induced hyperactivity (excitability, irritability and aggressiveness) in the present study represent animal experimental equivalents of psychotic side effects elicited in parkinsonian patients by dopamine substitution therapy, the results of the present studies suggest that the concurrent strong stimulation of both dopamine D_1 and D_2 receptors may be involved in the induction of some of the psychiatric side effects in pharmacotherapy with l-DOPA or apomorphine.

In the present study, the non-selective dopamine agonist apomorphine was suggested to have sufficient dopamine D_1 agonistic activity to ameliorate the symptoms of parkinsonism in the primate model under blockade of its D_2 agonist action, in contrast to SKF 38393 which deteriorated the parkinsonism (7), although apomorphine has been reported to exhibit a partial dopamine D_1 agonistic activity in the adenylate cyclase assay of rat striatal homogenates, like SKF 38393 (19). These discrepancies may be due to following: The dopamine D_1-receptor family, which is composed of D_1A and D_1B-receptor subtypes, is coupled to the stimulation of adenylate cyclase, but the conventional assay for the adenylate cyclase cannot detect differentially the intrinsic activity of some ligands on each subtype; and as a result, it is difficult at present to say whether apomorphine and SKF 38393 may act as a full or a partial agonist (possibly antagonist) to the D_1A- and D_1B-receptor subtypes, although they may bind with a high affinity to both subtypes (2). Thus, it can be assumed that apomorphine may be an agonist with a sufficient activity on the dopamine D_1-receptor subtype essential for the amelioration of the parkinsonism in the primate model, while SKF 38393 may be an antagonist.

Five subtypes of central dopamine receptors have been identified as described in the introduction, but these can be classified into two major families of receptors: dopamine D_1 (D_1A and D_1B, also known as D_1 and D_3, respectively) and dopamine D_2 (D_2A, D_2B and D_2C, also known as D_2, D_3 and D_4, respectively). Apomorphine and quinpirole have been shown to act as full agonists towards the dopamine D_2A-receptor subtype, while sulpiride acts as an antagonist (20); and they also bind with a high affinity to the dopamine D_2B and D_2C receptor, although it remains unclarified whether they may act as an agonist or an antagonist towards the dopamine D_2B and D_2C-receptor subtypes (21, 22). Moreover, the dopamine D_2B and D_2C receptors were considered to be involved in psychiatric symptoms in schizophrenic patients, because they were found to be rich in the mesolimbic and cortical areas of the brain, and the atypical neuroleptics such as clozapine and sulpiride bind with a high affinity to these receptors.

In conclusion, our findings suggest that stimulation of either central dopamine D_1 or dopamine D_2 receptors is requisite for the antiparkinsonian effects, and concurrent strong stimulation of both central dopamine D_1 and dopamine D_2 receptors causes marked hyperactivity that may be predictive of dopaminergic psychiatric side effects.

REFERENCES

7 Close SP, Marriott AS and Pay S: Failure of SKF 38393-A to relieve parkinsonian symptoms induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the marmoset. Br J Pharmacol 85, 320–322 (1985)
8 Nomoto M, Jenner P and Marsden CD: The D_1 agonist SKF 38393 inhibits the antiparkinsonian activity of the D_1 agonist...


17 Arnt J: Behavioral stimulation is induced by separate dopamine D1 and D2 receptor sites in reserpine-pretreated but not in normal rats. Eur J Pharmacol 113, 79–88 (1985)


19 Kebabian JW, Petzold GL and Greengard P: Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the "dopamine receptor". Proc Natl Acad Sci USA 69, 2145–2149 (1972)

