Effects on turning of microinjections into basal ganglia of D₁ and D₂ dopamine receptors agonists and the cannabinoid CB₁ antagonist SR141716A in a rat Parkinson’s model

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Brain cannabinoid CB₁ receptors are expressed in neural areas that contribute to movement such as basal ganglia, where they co-localize with dopamine D₁ and D₂ receptors. The objective of the present study was to further study the functional role of CB₁ receptors along with D₁ and D₂ dopamine receptors of basal ganglia by local injections of SR141716A (CB₁ receptor antagonist), SKF-38393 (D₁ agonist), and quinpirole (D₂ agonist), in a rat Parkinson’s model. Turning response after amphetamine was considered as the parkinsonian variable for quantifying motor effects of drugs. The findings indicated that, after intrastratial infusions, both D₁ or D₂ dopamine receptor agonists alone reduced turning in parkinsonian rats. At the pallidal and subthalamic intrastriatal infusions, both D₁ or D₂ dopamine receptor agonists alone reduced motor asymmetry in parkinsonian rats. At the pallidal and subthalamic levels, D₁ (not D₂) receptor stimulation also reduced rotation. Regarding SR141716A-induced effects, CB₁ antagonism reduced motor asymmetry in parkinsonian rats after injections into striatum, globus pallidus, and to a lesser extent, subthalamic nucleus. At the level of dorsal striatum, effects of SR141716A were mediated through an opposite modulation of D₁ and D₂ dopamine receptor function. At the pallidial and subthalamic nucleus levels, motor effects after SR141716A are not associated to modulation of D₁ and D₂ receptor function.

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Introduction

Brain cannabinoid CB₁ receptors are expressed in neural areas that contribute to movement such as the basal ganglia. Their density is high in subthalamic nucleus and dorsal striatum, where they co-localize with dopamine D₂ and D₁ receptors in projecting striatal neurons (Herkenham et al., 1990, 1991; Hermann et al., 2002; Surmeier et al., 1996; Tsou et al., 1998). The globus pallidus (GP) and substantia nigra pars reticulata (SNr) also contain a high density of CB₁ receptors, which are located on striatal and subthalamic nucleus terminals to these structures (Rodriguez de Fonseca et al., 1998). The cells in both GP and SNr are tonically active, and they serve to modulate the production of movement. Concretely, projections from internal GP and SNr to motor thalamus are the final output pathways in the basal ganglia circuit.

It is known that there is a continual intracerebral release of endogenous cannabinoid receptor agonists such as anandamide exhibiting neurotransmitter function (Baker et al., 2000; Giuffrida et al., 1999), and that the endocannabinoid system is an activity-dependent modulator of dopaminergic and glutamatergic neurotransmission in the basal ganglia (Beltramo et al., 2000; Cadogan et al., 1997; Gerdecman and Lovinger, 2001; Giuffrida et al., 1999; Glass et al., 1997; Gubellini et al., 2002; Pertwee, 1999). Endogenous cannabinoid CB₁ activation seems to act as a brake of dopaminergic and glutamatergic activity on dorsal striatum, and it has been proposed that it is a homeostatic counterregulatory mechanism in the basal ganglia (Rodriguez de Fonseca et al., 1994, 1998). However, local actions of cannabinoids suggest that they regulate neurotransmission in the basal ganglia in a more complex manner. Thus, local intranigral and intrastrial injections of exogenous cannabinoids induce contralateral rotation in intact rats indicating a higher functionality of the ipsilateral basal ganglia circuit, while intrapallidal and intrasubthalamic nucleus administrations of cannabinoids induce ipsilateral rotation (Miller et al., 1998; Sañudo-Peña et al., 1998a,b, 2000).

Although much is known about the central effects of exogenously applied cannabinoids, the function of endogenous cannabinoid systems needs further investigation. In this context, the discovery of the highly potent CB₁ receptor antagonist SR141716A has opened new possibilities for the identification and characterization of cannabinoid-dependent neuronal regulation. Concretely, its use has shown that there is a close interrelationship between CB₁ receptors and striatal dopamine D₁ and D₂ function (Rodriguez de Fonseca et al., 1994, 1998). Cannabinoid CB₁ antagonist-mediated effects within the striatum have been explained by the basal removing of the inhibitory influence of endogenous CB₁ receptor agonists on striatal dopamine D₂ recep-
tors (Giuffrida et al., 1999; Rodriguez de Fonseca et al., 1994, 1998), although the role of D1 receptors has not been discerned. Thus, the functional interaction between CB1 receptors and both D1 and D2 dopamine receptors also needs further investigation. These effects are of importance in light of the fact that degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to dopamine depletion in dorsal striatum, is the critical neuropathological and neurochemical correlate of Parkinson’s disease.

Motor effects of D1 and D2 agonists, along with CB1 ligands can be well studied by using the 6-OHDA-induced unilateral nigral lesion model of Parkinson’s disease in rats. Thus, D1 and D2 dopamineergic as well as CB1 cannabinoid receptors are known to be upregulated in denervated striata and corresponding globus pallidus (Fenton et al., 1984; Formaguera et al., 1994; Gnanalingham et al., 1995; Gower and Marriott, 1982; Lastres-Becker et al., 2001; Morelli and Di Chiara, 1987; Romero et al., 2000), so that motor effects of ligands of these receptors can be better discerned (Debonnel and de Montigny, 1988; Spooren et al., 1999). In the unilateral nigral lesion model, ipsilateral turning after amphetamine is caused by dopaminergic disbalance between lesioned and contralateral non-denervated basal ganglia circuit, hence changes in turning after local injections into basal ganglia of affected circuit are good indicators of functional changes in motor disbalance.

In the present study, SR141716A along with D1 and D2 dopamine receptor agonists have been tested in a rat Parkinson’s model based on the selective unilateral destruction of nigral neurons. This animal model parallels human disorder well, and produces a hemiparkinsonian syndrome which includes motor asymmetries, sensorimotor neglect, akinesia, and forepaw use deficit (Cenci et al., 2002; Formaguera et al., 1994; Marshall, 1979; Schwarting and Huston, 1996; Ungerstedt and Arbuthnott, 1970). This model has been extensively used to monitor the effects of grafts, neuroprotective agents, and the anti-akinetic potential of candidate antiparkinsonian drugs (Cenci et al., 2002; Schwarting and Huston, 1996). The local role of SR141716A and D1 and D2 receptors in dorsal striatum, external globus pallidus, and subthalamic nucleus, along with the possible interaction between CB1 antagonism and D1 and D2 receptors in basal ganglia, have been studied by means of local microinjections.

Materials and methods

Subjects

Male Wistar rats (275–325 g) from the breeding colony of the Faculty of Medicine of the University of Seville, Spain, were used. Laboratory temperature was kept at 22 ± 1°C, and a 12-h light–dark cycle (lights on at 08:00 h) was maintained throughout the experiment. Food (lab chow) and water were available ad libitum.

Unilateral 6-hydroxydopamine-induced nigra lesion

Thirty minutes before 6-hydroxydopamine (6-OHDA, RBI, USA) lesion, rats were injected with the antibiotic penicilnine (100,000 I.U. IM), and desipramine (Biolinck, Barcelona, Spain, 15 mg/kg IP) to protect noradrenergic terminals from 6-OHDA toxicity. Rats were anesthetized with chloral hydrate (425 mg/kg IP) and placed in a Kopf stereotaxic apparatus. Saline solution (1.2 μl per site) containing 6-OHDA (5 μg/μl, free base) and 0.2% ascorbic acid (Sigma, USA) was injected over 5 min with a blunted 30-gauge cannula at the following coordinates: AP = −5.2, −5.4, L = −2.2, and V = −8.2 mm (Paxinos and Watson, 1997). Control rats followed the same protocol except that the injected solution lacked 6-OHDA.

Guide cannula surgery, intracerebral microinjection and histology

Anesthetized rats were given prophylactic penicilnine and placed in a Kopf stereotaxic apparatus. A hole was drilled over the injection site, and a 22 gauge stainless steel guide cannula (Small Parts, USA) was aimed 2 mm above the corresponding infusion site: left dorsal striatum, AP = +0.5, L = −3, and V = −5.5; left external globus pallidus, AP = −0.8, L = −2.5, and V = −6.5; left subthalamic nucleus, AP = −3.7, L = −2.4, and V = −8.2 mm vs. bregma (Paxinos and Watson, 1997). The guide cannula was fastened to the skull with stainless steel screws and dental cement, and was fitted with a 30-gauge stainless steel obturator, protruding 1.5 mm out of the tip of the guide cannula (Small Parts). Injections were performed in the home cage after removing the obturator cannula that was replaced by a 30-gauge stainless steel internal cannula (Small Parts) connected to a Hamilton syringe and a delivery pump (Stoelting, Germany). Solutions were slowly injected over 5 min, and afterward, the internal cannula was carefully removed and the obturator cannula was replaced.

After completion of all the experiments, half of the experimental rats were anesthetized with chloral hydrate (425 mg/kg IP). Then, rats were humanely killed, brains were removed, and stored on 4% paraformaldehyde solution in phosphate buffer (PB) 0.1M (pH 7.2–7.4) at 4°C. Brains were sectioned (50 μm) with a vibratome, mounted on slides, and stained with cresyl violet (Nissl technique). Cannula placements were mapped onto a stereotaxic atlas (Paxinos and Watson, 1997) and confirmed to be in the left dorsal striatum, external globus pallidus, or subthalamic nucleus.

Chemicals and doses

SR141716A was gifted by Sanofi-Synthelabo Recherche (France). D-amphetamine and apomorphine were provided by RBI (USA). SR141716A was dissolved in 30% DMSO/70% distilled water. D-amphetamine dissolved in saline solution (0.9% NaCl). Apomorphine was dissolved in saline solution with 0.2% ascorbic acid. Regarding dopamine receptor ligands, SKF 38393 (D1 dopamine receptor agonist) was provided by Tocris (Barcelona, Spain), and dissolved in double distilled water. Quinpirole (D2 dopamine receptor agonist; RBI) was dissolved in 30% ethanol/70% distilled water.

For systemic injections, apomorphine was injected at 0 and 0.5 mg/kg SC; quinpirole at doses of 0, 4, and 6 mg/kg SC; and SKF38393 at 0, 2, and 4 mg/kg SC. For intracerebral infusions, SR141716A was injected at 0, 0.5, 1, and 1.5 μg/μl. For intracerebral infusions of dopaminergic ligands, SKF-38393 and quinpirole were each administered at 0, 0.5, and 1 μg/μl doses (free-base, SKF-38393, 0, 2.2, and 4.4 nmol; quinpirole, 0, 2.9, and 5.8 nmol). The most efficient SR141716A dose in each nuclei was selected for cotreatment with dopamine receptor ligands. The corresponding vehicle was used for the control group (dose 0) in every treatment. All drugs were injected at a
volume of 1 ml/kg body weight or at volumes of 1.5 μl (intrastratal), 1 μl (intrapallidal), and 0.5 μl (inrasubthalamic infusions).

Groups and general protocol

All parkinsonian rats presented a strongly positive response to amphetamine (>420 turns/h), 15 days after lesion. One week later, rats were subjected to microcannula implantation as explained, and different groups of parkinsonian rats were studied, each group with a cannula aimed at the left dorsal striatum (n = 15), external globus pallidus (n = 12), or subthalamic nucleus (n = 12). One week after microcannula surgery (1 month after nigral lesion), the CB1 antagonist SR141716A was administered directly through the cannula at several doses following a “Latin square” type design, and changing the initial dose for every rat, immediately before amphetamine (one test per day). One week after SR141716 alone injections, rats were treated with systemic apomorphine, quinpirole, and SKF38393 to discern if these dopaminergic agonists could induce contralateral rotation (one drug every 2 days). One week after the last systemic injection, the groups of rats were subjected to daily intracerebral injections of the two dopaminergic agonists quinpirole and SKF38393 alone or in combination with SR141716A, also following a “Latin-square” type design. Regarding intracerebral injections, all animals were treated with amphetamine immediately after intracerebral injections, and the percent change in number of amphetamine-induced turns with respect to animals with corresponding intracerebral vehicle injections was quantified.

Behavioral tests and statistics

For behavioral study, we followed a methodology previously described (Espejo et al., 1998; Fornaguera et al., 1994; Marshall, 1979; Schwarting and Huston, 1996; Ungerstedt and Arbuthnott, 1970). Thus, locomotor directional bias was evaluated by quantifying ipsiversive rotations induced by amphetamine (5 mg/kg IP). The number of ipsiversive rotations was quantified from 30 to 90 min after amphetamine (only those animals observed to make more than 420 turns/h were selected for the study, as explained). After subcutaneous injections of apomorphine, quinpirole, or SKF38393, the number of contralateral rotations was quantified from 5 to 65 min following injections, and the number of turns was statistically compared to control group (dose 0 or vehicle-treated animals) by using the Student’s t test.

Behavioral data after intracerebral microinjections of SR141716A or dopaminergic agonists alone were analyzed by using one-way ANOVA (dose as between variable) followed by post hoc Newman–Keuls test for comparison between dose groups. After cotreatment with SR141716A and dopamine receptor agonists, comparisons were made by using one-way ANOVA (treatment as between factor), followed by Newman–Keuls test for comparisons between two groups with different treatment. When variance was not homogeneous, data were logarithmically (log(x)) transformed before analysis. For statistical analysis, it was used the Crunch statistical program 3 (Crunch software corporation, Oakland, CA). Experiments were performed according the animal care guidelines of the European Communities Council (86/609/EEC).

Immunohistochemistry

To confirm the extent of the nigrostriatal lesions, half of the brains were immunostained against tyrosine-hydroxylase (TH). Rats were killed by decapitation and brains carefully removed. Brains were postfixed and stored in 4% paraformaldehyde in phosphate buffer (PB) 0.1 M (pH 7.2–7.4) at 4 °C, and immersed overnight in 25% sucrose in PBS for cryoprotection before sectioning. Coronal brain sections (50 μm thick) were cut on a cryostate and collected in PBS. Thereafter, endogenous peroxidase activity was quenched by placing sections into 0.3% H2O2 in 0.05 M Tris buffer (pH = 7.6) for 2 h. Then, sections were incubated in PBS/0.1% Triton X-100 (PBS-T) with 10% FCS (Vector, USA) and BSA (1 mg/mg, Sigma) for 4 h to block nonspecific sites. Sections were incubated overnight with rabbit anti-tyrosine-hydroxylase polyclonal antibody (anti-TH, 1:1000, Chemicon, USA) in PBS-T, and after washing in PBS-T, they were incubated for 1 h with anti-rabbit biotin-conjugated antibody (1:200, Chemicon). Then, sections were incubated with the ABC kit (1:100, Pierce, USA) in PBS-T, and after washing in PBS-T, they were incubated with amphetamine (>420 turns/h), 15 days after lesion. One week after SR14176 surgery (1 month after nigral lesion), the CB1 antagonist SR141716A was administered directly through the cannula at several doses following a “Latin square” type design, and changing the initial dose for every rat, immediately before amphetamine (one test per day). One week after SR141716 alone injections, rats were treated with systemic apomorphine, quinpirole, and SKF38393 to discern if these dopaminergic agonists could induce contralateral rotation (one drug every 2 days). One week after the last systemic injection, the groups of rats were subjected to daily intracerebral injections of the two dopaminergic agonists quinpirole and SKF38393 alone or in combination with SR141716A, also following a “Latin-square” type design. Regarding intracerebral injections, all animals were treated with amphetamine immediately after intracerebral injections, and the percent change in number of amphetamine-induced turns with respect to animals with corresponding intracerebral vehicle injections was quantified.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Dose (nmol)</th>
<th>Percent reduction in ipsilateral turning</th>
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<tbody>
<tr>
<td><strong>Dorsal striatum</strong></td>
<td></td>
<td></td>
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<tr>
<td>D1 receptor agonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKF 38393</td>
<td>2.2</td>
<td>39 ± 8*</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>47.7 ± 7*</td>
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<tr>
<td>D2 receptor agonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinpirole</td>
<td>2.9</td>
<td>73 ± 5**</td>
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<tr>
<td></td>
<td>5.8</td>
<td>69 ± 9**</td>
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<tr>
<td><strong>Globus pallidus</strong></td>
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<tr>
<td>D1 receptor agonist</td>
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<tr>
<td>SKF 38393</td>
<td>2.2</td>
<td>47 ± 14*</td>
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<td></td>
<td>4.4</td>
<td>49.2 ± 8.2*</td>
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<tr>
<td>D2 receptor agonist</td>
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<tr>
<td>Quinpirole</td>
<td>2.9</td>
<td>22.2 ± 7*</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
<td>12.1 ± 7*</td>
</tr>
<tr>
<td><strong>Subthalamic nucleus</strong></td>
<td></td>
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<tr>
<td>D1 receptor agonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKF 38393</td>
<td>2.2</td>
<td>28 ± 4*</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>31 ± 7*</td>
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<tr>
<td>Outside the nucleus</td>
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<tr>
<td></td>
<td>2.2</td>
<td>0</td>
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<tr>
<td></td>
<td>4.4</td>
<td>0</td>
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<tr>
<td>D2 receptor agonist</td>
<td></td>
<td></td>
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<tr>
<td>Quinpirole</td>
<td>2.9</td>
<td>25.2 ± 8</td>
</tr>
<tr>
<td></td>
<td>5.8</td>
<td>1 ± 3</td>
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<tr>
<td>Outside the nucleus</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2.9</td>
<td>0</td>
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<tr>
<td></td>
<td>5.8</td>
<td>0</td>
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</tbody>
</table>

Mean ± SEM.
* P < 0.05 vs. vehicle-treated rats (0 dose, percent value = 0; Newman–Keuls test).
** P < 0.01 vs. vehicle-treated rats (0 dose, percent value = 0; Newman–Keuls test).
brain sections, with the exception that the primary antibody solution was replaced by a PBS-T solution containing 10% FCS and BSA (1 mg/ml) without the primary antibody. Sections were washed in PBS and mounted on glass slides and coverslipped with DPX. The location of cannula tip was also verified in the immunostained coronal sections. Cannula placements were mapped onto a stereotaxic atlas (Paxinos and Watson, 1997) and confirmed to be in the left dorsal striatum, external globus pallidus, or subthalamic nucleus.

Results

Local injections of D1 and D2 dopamine receptor agonists into dorsal striatum, external globus pallidus, and subthalamic nucleus

First, parkinsonian rats presented a significant number of contralateral rotations after 0.5 mg/kg apomorphine (320 ± 87 turns per hour, t = 3.7, P < 0.01 vs. control). Besides, a significant number of contralateral turnings was also observed after systemic quinpirole (4 mg/kg, 68.8 ± 32, turns/h, t = 2.5, P < 0.05; 6 mg/kg, 290 ± 34 turns/h, t = 3.4, P < 0.01) and SKF38393 (2 mg/kg, 334 ± 88 turns/h, t = 3.8, P < 0.01; 4 mg/kg, 450 ± 55 turns/h, t = 4.7, P < 0.01), indicating that these agonists of D1 and D2 dopamine receptors could induce contralateral rotation after systemic injections.

To better discern the role of D1 and D2 dopamine receptors on motor asymmetry of parkinsonian rats (as measured through turning after amphetamine), SKF-38393 and quinpirole were injected into left basal ganglia nuclei (side of dopaminergic lesion): denervated dorsal striatum, globus pallidus, and subthalamic nucleus. Regarding intrastriatal injections, both dopaminergic agonists reduced turning after amphetamine vs. vehicle-treated rats, indicating an amelioration of parkinsonian motor asymmetry (Table 1). Thus, turning was significantly reduced after both doses of SKF-38393 (F(2,44) = 3.4, P < 0.05) and quinpirole (F(2,44) = 3.3, P < 0.05), as shown in Table 1. For intrapallidal injections, only SKF-38393 significantly reduced amphetamine-induced turning in a dose-dependent manner, as revealed by a dose effect in ANOVA (F(2,35) = 3.4, P < 0.05), indicating that D1, but not D2, dopamine receptors of the external globus pallidus are involved in dopamine-mediated effects after

Fig. 2. Percent reduction in ipsilateral turning after intra- striatal treatment with SR141716A (1.5 µg/µl), dopaminergic agonists and combination of both treatments (injections in the side of the dopaminergic lesion, striatal, n = 15, pallidum, n = 12, subthalamic nucleus, n = 9). Mean ± SEM, *P < 0.05, **P < 0.01 vs. corresponding 0 dose group. Abbreviation: SR, SR141716A; SKF0.5, SKF38393 0.5 µg/µl (2.2 mmol); SKF1, SKF38393 1 µg/µl (4.4 mmol); Qui0.5, quinpirole 0.5 µg/µl (2.9 mmol); Qui1, quinpirole 1 µg/µl (5.8 mmol).
amphetamine on motor asymmetries. All intrastriatal and intrapallidal injections were found to be located inside the nucleus. Finally, regarding intrasubthalamic injections, SKF-38393 significantly reduced turning \( (F(2,26) = 3.1, \ P < 0.05) \) in a dose-dependent manner, but quinpirole was devoid of significant motor effects. Three injections were found to be located in zona incerta, outside the subthalamic nucleus, but this location did not modify turning in comparison with vehicle-treated rats, as shown in Table.

Fig. 4. Locations of infusions into left dorsal striatum, external globus pallidus and subthalamic nucleus (from left to right) in parkinsonian rats that received local injections (the border of the nucleus is drawn with a dashed line). Bottom row: pictures of representative sites of injections in coronal brain sections stained with Nissl technique. Rats with cannula tip locations outside the subthalamic nucleus were discarded for statistical analysis. Plates are taken from Paxinos and Watson (1997). Abbreviation: Str, dorsal striatum; GP, globus pallidus; STh, subthalamic nucleus.
Local injections of SR141716A into dorsal striatum, external globus pallidus, and subthalamic nucleus

After left intrastriatal, intrapallidal, and intrasubthalamic injections, one-way ANOVA revealed significant dose effects (dorsal striatum, F(3,42) = 6.8, P < 0.05; external globus pallidus, F(3,33) = 11.4, P < 0.01; subthalamic nucleus, F(3,24) = 5.9, P < 0.05), as shown in Fig. 1. Regarding intrastriatal injections, amphetamine-induced turning was significantly reduced following 1 (P < 0.05 vs. 0 dose) and 1.5 μg/μl SR141716A (P < 0.01). For intrapallidal infusions, turning was reduced in a dose-dependent manner following every SR141716A dose (P < 0.01 vs. 0 dose). After intrasubthalamic injections, a significant reduction of turning was only observed at 1 μg/μl SR141716A dose (P < 0.05 vs. 0 dose), and changes followed a U-shaped curve. No changes on turning were observed in rats with local injections outside the subthalamic nucleus. These data indicated that SR141716A dose-dependently improves motor asymmetries after injections into denervated striatum and globus pallidus, and its effect is not dose-dependent in the subthalamic nucleus.

Interaction of SR141716A with intrastratal dopamine receptors: opposite modulation of D₁ and D₂ receptor function

One-way ANOVA indicated significant treatment effects after local infusions of the D₁ dopamine receptor agonist in combination with SR141716A (4.8 nmol, 1.5 μg/μl, dose with maximum effect), as shown in Fig. 2 (treatment effect, F(4,74) = 6.2, P < 0.01). Post hoc analyses revealed that SKF-38393 (D₁ receptor agonist, 2.2 and 4.4 nmol) in combination with SR141716A had significantly greater functional effects than SR141716A and SKF-38393 given alone (P < 0.01). This finding points to a positive modulation of SR141716A of motor effects after stimulation of D₁ dopamine receptors. On the other hand, one-way ANOVA also indicated significant treatment effects after local infusions of the D₂ dopamine receptor agonist in combination with SR141716A (4.8 nmol, 1.5 μg/μl, dose with maximum effect), as shown in Fig. 2 (treatment effect, F(4,74) = 6.4, P < 0.01). Thus, quinpirole (D₂ receptor agonist, 2.9 and 5.8 nmol) in combination with SR141716A had significantly lower functional effects than SR141716A and quinpirole given alone. This finding suggests that SR141716A negatively modulates motor effects following D₂ dopamine receptor stimulation.

Absence of interaction of SR141716A with intrapallidal and intrasubthalamic D₁ and D₂ dopamine receptor function

After intrapallidal injections of dopamine receptor agonists in combination with SR141716A (1.5 μg/μl), nonsignificant treatment effects were observed as shown in Fig. 3, revealing that pallidal D₁ and D₂ receptors are not involved in SR141716A-induced effects on motor output. Regarding intrasubthalamic injections, no significant effects of treatment were found after infusions of dopamine receptor agonists in combination with SR141716A (data not shown), indicating that there is no interaction of the cannabinoid CB₁ receptor antagonist with D₁ or D₂ receptor function in the subthalamic nucleus.

Local injections: histology

Only those animals, where injection site was found to be correct, were included for analyses. Fig. 4 illustrates the central cannula tip location in left dorsal striata, external globus pallidus, and subthalamic nucleus in hemiparkinsonian rats, as well as representative pictures of coronal sections stained with the Nissl technique. Inspection of brain tissue revealed evidence of a small lesion and gliosis at the site of injection, although surrounding tissue was generally intact.

Discussion

Role of D₁ and D₂ dopamine receptor of several basal ganglia on parkinsonian motor asymmetry

It is quite interesting that agonists of either D₁ or D₂ dopamine receptors ameliorated motor asymmetries in parkinsonian rats after infusions into the denervated dorsal striatum, and that the same dopaminergic agonists induced contralateral rotation after systemic injections, further pointing to a dopaminergic disbalance between both hemispheres. In this context, D₁ and D₂ dopamine receptors are mostly segregated in two different populations of GABA striatal neurons which lead to the two output pathways: direct pathway (GABA neurons expressing D₁ receptors), projecting to internal globus pallidus and substantia nigra, and indirect pathway (GABA neurons expressing D₂ receptors), projecting to external globus pallidus (Keefe and Gerfen, 1995; McKenzie et al., 1984; Nicola et al., 2000; O’Connor, 1998; Onn et al., 2000; Pan et al., 1992; Svendsen et al., 2000). From a functional point of view, stimulation of either D₁ or D₂ receptors would resemble physiological effects of dopamine, leading to a net reduction of the abnormal hyperactivity of internal pallidum that is characteristic of denervated dopaminergic basal ganglia circuit in Parkinson’s disease (Löschmann et al., 1997; Onn et al., 2000). This finding supports the notion that dopamine agonists acting at D₁ or D₂ receptors are of value for treatment of parkinsonian symptoms (Olanow, 1992; Watts and Koller, 1997).

At the pallidal level, only D₁ receptor stimulation led to a reduction of ipsilateral turning after amphetamine. Stimulation of pallidal D₂ receptors was devoid of effects. Pallidal neurons are known to express both D₁ and D₂ receptors (Carlson et al., 1988; Pan et al., 1990), and intrapallidal dopamine restores up to 50% of the motor performance in the 6-OHDA rat model (Galvan et al., 2001). The findings of the present study suggest that D₁ dopamine receptors would be those involved in the beneficial effects of dopaminergic stimulation, exerting a positive influence on motor output of basal ganglia in parkinsonian rats. This result is also in line with the production of akinesia after blockade of D₁ receptors in the globus pallidus (Hauber and Lutz, 1999).

Regarding subthalamic nucleus infusions, D₁ (not D₂) receptor stimulation led to a reduction of motor asymmetries. This is an interesting result, further confirming findings reported by others (Hassani and Feger, 1999). Thus, in 6-hydroxydopamine-lesioned rats, the hyperactivity of subthalamic nucleus is reduced by local infusions of D₁ receptor agonists but not D₂ dopamine agonists (Hassani and Feger, 1999). Hyperactivity of the subthalamic nucleus is a critical feature in the pathophysiolog of Parkinson’s disease. Hence, reducing firing activity after D₁ receptor stimulation would ameliorate motor deficits, as revealed by a reduction in the rotational...
response of parkinsonian rats. It has been proposed that dopamine agonists would exert a therapeutic effect at the level of the subthalamic nucleus by decreasing subthalamic nucleus activity via stimulation of D2 receptors (Albin et al., 1989). Our data do not support a role for D2 receptors on the regulation of subthalamic nucleus activity in parkinsonian rats, in accordance with Kreiss et al. (1997) who found that stimulation of D2 receptors in the subthalamic nucleus with systemic quinpirole alone was ineffective except if a D1 agonist was administered before. Curiously, systemic administration of D1 receptor agonist enhances subthalamic neuronal firing in parkinsonian rats hence worsening basal ganglia function (Kreiss et al., 1997), suggesting that systemic administration alters activity in the subthalamic nucleus in an opposite way to that after intrasubthalamic injections. This result is likely a consequence of firing changes in other basal ganglia areas connected with the subthalamic nucleus, such as pallidousubthalamic or nigrosubthalamic inputs, or it could be accounted for by the participation of the contralateral subthalamic nucleus after systemic injections.

Excitatory action of local SR141716A on basal ganglia of parkinsonian rats: differential role of D1 and D2 receptors

It is known that local actions of cannabinoid agonists suggest that they regulate neurotransmission in the basal ganglia in a complex manner. Thus, local intrastriatal injections of exogenous cannabinoids induce contralateral rotation in intact rats indicating a motor disbalance favoring functional action of the ipsilateral basal ganglia circuit, while intrapallidal and intrasubthalamic nucleus administrations of cannabinoids induce ipsilateral rotation, pointing to an inverse effect to that of intrastriatal injections (Miller et al., 1998 San˜udo-Pena et al., 1998b, 2000).

In the present study, ipsilateral rotation in hemiparkinsonian rats was reduced after infusions of a cannabinoid receptor antagonist into denervated striata, indicating an improvement of motor performance. The results suggest that SR141716A acted through an opposing action on D1 and D2 receptor function, leading to a positive modulation of motor processes induced by D1 receptor stimulation, but to a reduction of D2 dopamine receptor function. SR141716A-induced effects on striatal dopamine receptors could be indirect or through receptor-receptor interaction, a fact which deserves further investigation. The findings are different to those found with intrastriatal cannabinoid agonists, where dopamine agonists (acting at either D1 or D2 receptors) blocked cannabinoid-induced effects in denervated striata (San˜udo-Pena et al., 1996). This fact indicates that SR141716A-mediated effects in denervated striata rely on different mechanisms to those after CB1 receptor agonism, since SR141716 positively or negatively modulates motor processes after D1 and D2 receptor stimulation, respectively, but CB1 agonists seem to stimulate motor effects induced by stimulation of both D1 and D2 receptors. Cannabinoid CB1 antagonist-mediated effects within the striatum have been explained by the basal removing of the inhibitory influence of endogenous CB1 receptor agonists on striatal dopamine receptors (Giuffrida et al., 1999; Rodrigue de Fonseca et al., 1994, 1998), and the data of the present study support that motor effects after CB1 antagonist infusion into denervated striata rely on a positive modulation of functional effects after stimulation of D1 receptors, but also points to a negative modulation of functional effects after D2 receptor stimulation. In this context, Alonso et al. (1999) have reported that SR141716A reduce D2 dopamine function in the striatum.

On the other hand, intrapallidal infusions of SR141716A in parkinsonian rats also reduced ipsilateral turning after amphetamine. D1 and D2 dopamine receptors seem not to participate in this effect, which is in contrast with motor effects found after coinfusions of cannabinoid agonists and dopamine ligands in the globus pallidus, where D2 receptor agonists block cannabinoid effects (San˜udo-Peña and Walker, 1998). Again, this result indicates that intrapallidal effects of CB1 antagonism after SR141716A rely on different mechanisms to those after CB1 receptor stimulation, where D2 receptors appear to be involved. An improvement of motor function after intrapallidal injections of SR141716A is in accordance with the fact that augmented levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in parkinsonian rats (DiMarzo et al., 2000).

Finally, effects of SR141716A on subthalamic nucleus were less stronger, and only a moderate dose reduced ipsilateral rotation after amphetamine. Changes followed a U-shaped curve, a finding difficult to explain. In this context, the U-shaped curve could suggest the existence of two different populations or subtypes of cannabinoid receptors in the subthalamic nucleus since this type of curve can be described by two exponential function (Meuth et al., 2002). D1 and D2 dopamine receptors do not seem to participate in this effect. Considering the small size of the subthalamic nucleus, it is possible that effects could be also due to diffusion to the nearby zona incerta, but this is unlikely because the injection volume was small and infusions into adjacent areas did not give changes on rotation.

Conclusions

The findings of the present study indicated that, after intrastriatal infusions of dopamine agonists alone, both D1 and D2 dopamine receptor agonists reduced motor asymmetries in hemiparkinsonian rats. Intrapallidal and intrasubthalamic injections of dopamine agonists revealed that only D1 dopamine receptor stimulation led to reduction of rotation. Local SR141716A, CB1 receptor antagonist, reduced motor asymmetries after injections into dorsal striatum, external globus pallidus, and to a lesser extent, subthalamic nucleus. At the level of dorsal striatum, effects of SR141716A were mediated by an opposite modulation of D1 and D2 dopamine receptor function, enhancing motor processes mediated by stimulation of D1 receptors. At the pallidal and subthalamic nucleus levels, dopamine receptors do not seem to participate on motor effects after SR141716A.

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References

SR141716A selectively increases FOS expression in rat mesocorticolimbic areas via reduced dopamine D2 function. Neuroscience 91, 607 – 620.


Hauber, W., Lutz, S., 1999. Dopamine D1 or D2 receptor blockade in the globus pallidus produces akinesia in the rat. Brain Res. 106, 143 – 150.


