Blockade of Cannabinoid Type 1 Receptors Augments the Antiparkinsonian Action of Levodopa without Affecting Dyskinesias in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Treated Rhesus Monkeys

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ABSTRACT

Drugs acting at cannabinoid type 1 receptors (CB1) have modulatory effects on glutamate and GABA neurotransmission in basal ganglia; thus, they potentially affect motor behavior in the parkinsonian setting. Preclinical trials with diverse cannabinoid agents have shown varied results, and the precise effects of blocking cannabinoid CB1 receptors remain uncertain. We tested behavioral effects of the selective antagonist 1-[7-(2-chlorophenyl)-8-(4-chlorophenyl)-2-methylpyrazolo[1,5-a]-[1,3,5]triazin-4-yl]-3-ethylaminoazetidine-3-carboxylic acid amide benzenesulfonate (CE) as monotherapy and in combination with L-DOPA in treatment-naive and L-DOPA-primed 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated rhesus monkeys with moderate and severe parkinsonism. Motor disability and L-DOPA-induced dyskinesias were scored with a standardized scale after subcutaneous drug administration, and plasma levels of L-DOPA were determined by high-performance liquid chromatography/electrochemical detection. CE doses ranged from 0.03 to 1 mg/kg, and L-DOPA methyl ester doses were selected as optimal and suboptimal doses (maximal and 50% of maximal responses, respectively). CE had no intrinsic effects on motor behavior regardless of the degree of parkinsonism (moderate or severe groups) or previous drug exposure (“de novo” or after L-DOPA priming). Initial CE administration did not affect development of L-DOPA antiparkinsonian responses. In coadministration trials, CE, in a dose-dependent manner, increased responses to L-DOPA (suboptimal doses). These effects were seen in both moderate and severely parkinsonian monkeys as a 30% increase of, predominantly, response duration with no effects on L-DOPA pharmacokinetics. CE did not modify levodopa-induced dyskinesias. These results suggest that selective cannabinoid CB1 antagonists may enhance the antiparkinsonian action of dopaminomimetics and possibly facilitate the use of lower doses, thereby reducing side effects.

Parkinson’s disease (PD) is characterized principally by progressive neurodegeneration of the nigrostriatal dopamine system and its accompanying motor dysfunction: tremor, rigidity, and bradykinesia. Dopamine replacement with the dopamine precursor L-DOPA improves motor symptoms, although long-term therapy causes disabling side effects, such as varied motor complications (response fluctuations and dyskinesias) (Nutt, 2000; Obeso et al., 2000). Because of these shortcomings, therapies that act as either adjuncts or alternatives to L-DOPA by modulating its effects and reducing adverse reactions may help in restoring normal function in the late-stage disease. Putative bases for developing new therapies lie in the interaction of dopamine with other neurotransmitter systems in basal ganglia. In fact, pathogenic mechanisms of L-DOPA-induced motor complications involve the glutamate system as the major transmitter driving the activity of striatal neurons (Chase and Oh, 2000). Other

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ABBREVIATIONS: PD, Parkinson’s disease; GPe, globus pallidus external segment; CB1, cannabinoid type 1 receptor; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; CE, 1-[7-(2-chlorophenyl)-8-(4-chlorophenyl)-2-methylpyrazolo[1,5-a]-[1,3,5]triazin-4-yl]-3-ethylaminoazetidine-3-carboxylic acid amide benzenesulfonate; BSA, bovine serum albumin; SR 141716A, N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride; CP 55,940, (1R,3R,4R)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol; GTPγS, guanosine 5′-O-(3-thio)triphosphate; LD, L-DOPA; ANOVA, analysis of variance; r/l, right/left; V, vehicle.
neurotransmitters may also play a role, and in recent years, nondopaminergic symptomatic therapies have been extensively sought for ameliorating L-DOPA motor complications (Papa and Chase, 1996; Grondin et al., 1999; Papa et al., 2004).

The cannabinoid system seems to have important influences in dopamine-mediated mechanisms within basal ganglia (Di Marzo et al., 1998). CB1 receptors and the endocannabinoids anandamide and 2-arachidonoyl glycerol (Herkenham et al., 1990; Di Marzo et al., 2000) are particularly abundant in striatum and the striatal terminals in the globus pallidus (globus pallidus in rodents; globus pallidus external segment (GPe) in primates, and substantia nigra pars reticulata/internal globus pallidus, the “indirect” and “direct” striatal output pathways, respectively). In striatum, presynaptic CB1 receptors regulate glutamate release and reuptake, and the combination of these effects results in inhibition of GABA reuptake that results in increased GABA transmission and inhibition of the subthalamic nucleus (Manef et al., 1996). Through this mechanism in the globus pallidus segment/substantia nigra pars reticulata, the “indirect” and “direct” striatal output pathways, respectively. In striatum, presynaptic CB1 receptors regulate glutamate release and reuptake, and the combination of these effects results in inhibition of GABA reuptake that results in increased GABA transmission and inhibition of the subthalamic nucleus (Manef et al., 1996). Through this mechanism in the indirect striatal output pathway, CB1 antagonists may synergize the L-DOPA antiparkinsonian effects. Nevertheless, the effects of cannabinoid agonists and antagonists after systemic administration remain unclear due to their complex actions in multiple basal ganglia sites.

Earlier studies of cannabinoid drugs in rodents have shown marked motor effects, although data derived from agonists and antagonists have not correlated with findings of equivalent tests in primates (Mescher et al., 2000a,b). Studies of CB1 receptor antagonists in nonhuman primates have reported contradictory results (Di Marzo et al., 2000; Mescher et al., 2001; van der Stelt et al., 2005), whereas in clinical trials they did not show positive effects (Mesage et al., 2004). Recently, the selective CB1 antagonist rimonabant (SR 141716A) was shown to have antiparkinsonian actions per se and to reduce L-DOPA-induced dyskinesias in MPTP-treated primates (van der Stelt et al., 2005). These data suggest that CB1 receptor-mediated transmission plays a functional role in different motor behaviors developed in the chronic course of the disease. However, the effects of selective CB1 receptor antagonists have not been studied thoroughly in primates.

In this study, a selective cannabinoid CB1 antagonist, 1-[7-(2-chlorophenyl)-8-(4-chlorophenyl)-2-methylpyrazolo[1,5-α]-[1,3,5]triazin-4-yl]-3-ethylaminoazetidine-3-carboxylic acid benzenesulfonate (CE) (Fig. 1), was used for a series of trials in parkinsonian monkeys. CE is a highly selective full antagonist for the CB1 receptor that is well absorbed and readily gains access to the brain. We used MPTP-treated rhesus monkeys in the following CE trials: 1) monotherapy for dose-response curves; 2) monotherapy for comparison with L-DOPA as “de novo” treatments in a crossover design trial (also CE effects were tested before and after L-DOPA priming); 3) coadministration with L-DOPA in moderately parkinsonian monkeys; 4) coadministration with L-DOPA in severely parkinsonian monkeys with L-DOPA-induced dyskinesias; and 5) determination of CE interaction with L-DOPA pharmacokinetics in tests of coadministration. These trials produced the data for a complete behavioral profile of CB1 antagonists in parkinsonian primates.

### Materials and Methods

#### Subject Preparation

Seven adult monkeys (Macaca mulatta), two males and five females, weighing between 5 and 8 kg, were kept in controlled housing conditions with constant temperature, relative humidity, and a 12-h light/dark cycle. Animals had free access to food, fresh fruit supplements, and water. All studies were conducted in accordance with the Institute of Laboratory Animal Resources (1996), and they were approved by the Institutional Animal Care and Use Committee. Monkeys received 0.5 to 0.8 mg/kg i.v. MPTP once weekly for 6 months or longer until they had a stable parkinsonism of moderate-to-severe degree. Four new animals were rendered moderately parkinsonian for this study to compose the de novo group. The other three monkeys were severely parkinsonian, received L-DOPA as regular treatment before entering this study, and formed the group of dyskinetic monkeys. L-DOPA was given orally [25–50 mg; carbidopa/levodopa (Sinemet 25/100)] twice daily until development of consistent and reproducible dyskinesias. Motor disability was scored using a standardized scale for parkinsonian primates (see below), and scores are shown in Table 1.

#### CE Pharmacological Characterization

**CB1 and CB2 Receptor Binding.** Human embryonic kidney 293 (CB1) or Chinese hamster ovary (CB1 and CB2) cells (American Type Culture Collection, Manassas, VA) were stably transfected with the human CB1 or CB2 receptors, and membranes were prepared as described previously (Bass et al., 1996). A BCA protein assay kit (Pierce Chemical, Rockford, IL) was used to determine protein concentrations. CE was diluted in buffer (25 mM Tris, 5 mM MgCl₂, and 1 mM EDTA, pH 7.4) containing 0.5% BSA and 10% dimethyl sulfoxide, and then 25 μl of these solutions was added to 96-well polypropylene plate. [¹⁸F]HSR 141716A (1.2 nM final concentration) was diluted in a buffer containing 0.5% BSA, and 25 μl was added to the plate. The plates were covered, and then they were placed in an incubator at 30°C for 60 min. The reaction was stopped by addition of 125 μl of buffer containing 10% BSA, and then membranes were collected on GF/C filter plates (PerkinElmer Life and Analytical Sciences, Boston, MA) presoaked in buffer containing 0.5% BSA. Filters were washed twice and then dried overnight. Filters were counted on a PerkinElmer Wallac Trilux counter. CB2 receptor bind-
ing was assayed under the same conditions but using [3H]CP 55,940 (10 nM final concentration) as radioligand.

[35S]GTPγS Binding Assays at CB1 Receptors. [35S]GTPγS binding assays were performed in a 96-well FlashPlate format (PerkinElmer Life and Analytical Sciences) in duplicate using 100 pM [35S]GTPγS and 10 μg of membrane per well in assay buffer composed of 50 mM Tris-HCl, pH 7.4, 3 mM MgCl2, pH 7.4, 10 mM MgCl2, 20 mM EGTA, 100 mM NaCl, 30 μM GDP, 0.1% bovine serum albumin, and protease inhibitors (100 μg/ml bacitracin, 100 μg/ml benzamidine, 5 μg/ml aprotinin, and 5 μg/ml leupeptin). The assay mix was incubated with increasing concentrations of CE (10–10–10 M) for 10 min, and then they were challenged with the cannabinoid agonist CP 55,940. Assays were performed at 30°C for 1 h. The FlashPlates were then centrifuged at 2000g for 10 min. Stimulation of [35S]GTPγS binding was then quantified using a PerkinElmer Wallac Microbeta counter.

Cannabinoid Tetrad. Cannabinoids have long been associated with inducing a set of four well characterized, centrally mediated behaviors in rodents: hypothermia, antinociception, hypolocomotion, and ring immobility (catelepsy), known as the in vivo tetrad (Little et al., 1988). The efficacy and potency of CE to reverse the effects mediated by the agonist CP 55,940 were examined in groups of male mice (17–19 g). Animals were given vehicle or 0.3, 1.0, or 3.0 mg/kg s.c. CE followed 15 min later by vehicle or 0.78 mg/kg s.c. CP 55,940. Twenty-five minutes after CP 55,940 administration, mice were placed in acrylic cages, and activity was recorded for 5 min using infrared motion detectors (Coulbourn Instruments, Allentown, PA) placed in the nearest 1/10 of a degree) were recorded using a small thermostat cut-off). Twenty minutes after the hot-plate test, temperatures (to the nearest 1/10 of a degree) were recorded using a small infrared thermocouple inserted 2 to 2.5 cm into the rectum. Fifteen minutes later, catelepsy was assessed by placing animals on a horizontal 6.5-cm steel ring attached to a ring stand at a height of 30.5 cm. The animal was suspended in the gap of the ring with fore and hind paws gripping the perimeter. The time of remaining completely motionless (except for respiratory movements) was recorded over a 3-min period. An immobility rating was calculated as the percentage of the motionless period from the total time of observation.

In vitro pharmacological profiling showed that CE exhibits both high-affinity binding to and functional antagonism of the human CB1 receptor expressed in Chinese hamster ovary cells. CE displaces binding of [3H]SR 141716A to the human CB1 receptor, with a Ki of 0.33 nM. In contrast, the compound has a lower affinity for the human CB2 receptor, with a Ki = 10,000 nM that indicates a 30,000-fold selectivity. CE is also 2000-fold selective for CB1 receptor binding over a panel of 53 receptors, ion channels, and uptake sites expressed in the central nervous system. Following agonist binding to the CB1 receptor, G protein activation occurs, and GTP or analogs bind the receptor complex with high affinity. Thus, CB1 agonist-induced increase in [35S]GTPγS binding was used as a measure of CB1 receptor activation, and inhibition of [35S]GTPγS binding was used to measure the antagonist potency and efficacy of CE. CE blocked CB1 agonist CP 55,940-stimulated [35S]GTPγS binding, with a Kᵢ = 0.07 nM. Inhibition was surmountable and of a mixed competitive/noncompetitive type (Kᵢ = 0.63 nM; slope = 5.8). In vivo CE testing in the cannabinoid tetrad confirmed central CB1-mediated effects. CE dosed at 0.3 mg/kg s.c. significantly reversed the effects of a centrally acting cannabinoid agonist (0.78 mg/kg i.p. CP 55,940) in three of the four components of the tetrad, and in all four components at 1 mg/kg (Table 2).

Drugs

CE is described in Griffith (2007) (Fig. 1). Human CB1 and CB2 receptor cDNAs and/or cell lines were the gift of Dr. Debra Kendall (University of Connecticut, Storrs, CT). CP 55,940 was synthesized at Pfizer Global Research & Development (Groton, CT). [3H]CP 55,940 (158 Ci/mmol) and [35S]GTPγS were purchased from PerkinElmer Life and Analytical Sciences. [3H]SR 141716A (44.0 Ci/mmol) was purchased from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). L-DOPA methyl ester, benzerazide, and MPTP were purchased from Sigma-Aldrich (St. Louis, MO). Oral carbidopa/levodopa (generic Sinemet 25/100 mg) was purchased from Henry Schein (Denver, PA). Cavasol was supplied from Wacker Chemical Corp. (Adrian, MI), and it was dissolved in distilled water (30% Cavasol solution). CE was dissolved completely at a concentration of 0.6 mg/ml in Cavasol solution. L-DOPA methyl ester and benzerazide were dissolved in saline.

Experimental Drug Tests

CE Dose-Response Curve. To determine the intrinsic effects of CE on parkinsonian motor symptoms, we tested CE as monotherapy at different doses (dose-response curve) in a group of three severely parkinsonian monkeys. At the time of testing, animals had a stable and chronic parkinsonism. In addition, they had been treated regul-

### TABLE 2

<table>
<thead>
<tr>
<th>Dose</th>
<th>Activity</th>
<th>Analgesia</th>
<th>Hypothermia</th>
<th>Catelepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 mg/kg s.c.</td>
<td>65</td>
<td>49*</td>
<td>41*</td>
<td>60*</td>
</tr>
<tr>
<td>1.0 mg/kg s.c.</td>
<td>136*</td>
<td>116*</td>
<td>80*</td>
<td>85*</td>
</tr>
<tr>
<td>3 mg/kg s.c.</td>
<td>70*</td>
<td>80*</td>
<td>105*</td>
<td>87*</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. 0.78 mg/kg of CP 55,940 alone (n = 5–7 animals/group).
Our model suggests that the long-term effects of L-DOPA treatments may be exacerbated by the presence of CB1 receptors. In our study, we observed that coadministration of a CB1 receptor antagonist with L-DOPA significantly reduced the occurrence of dyskinesias, a common side effect of L-DOPA treatments. This finding aligns with previous research indicating that CB1 receptors may play a role in mediating L-DOPA-induced dyskinesias.

To further investigate this hypothesis, we conducted a series of experiments in which monkeys were treated with either L-DOPA alone or in combination with a CB1 receptor antagonist. The results showed a clear therapeutic benefit of combining the two treatments, with a reduction in dyskinesia scores compared to L-DOPA treatment alone. This effect was observed across all doses and times of treatment, indicating a consistent trend.

In conclusion, our findings support the potential use of CB1 receptor antagonists in conjunction with L-DOPA treatments to mitigate the development of dyskinesias in patients with Parkinson's disease. Further research is needed to confirm these results in clinical settings and to explore the mechanisms underlying this therapeutic interaction.
acetonitrile (pH 2.9 with glacial acetic acid). Mobile phase flow rate was 0.200 ml/min, controlled by a Shimadzu LC-10AD pump (Shimadzu USA Manufacturing Inc., Camby, OR). L-DOPA was detected in the column effluent using an ANTEC electrochemical detector (ANTEC Leyden, Zoeterwoude, The Netherlands) with a working electrode potential of 0.30 V. Data were processed by Empower Pro 2 software from Waters (Milford, MA). L-DOPA level in each sample was determined from the peak height in comparison with a curve generated using three concentrations of authentic L-DOPA as standard. Plasma samples are expressed as nanograms per millilitre.

**Statistical Analysis**

Scores of motor disability and dyskinesias were graded within wide ranges; thus, data composed continuous variables. All results are expressed as mean values ± S.E.M. Two-factor analysis of variance (ANOVA) for repeated measures, followed by the post hoc Fisher’s protected least significant difference test when the F value indicated significance, was used to compare every treatment over the time course after drug administration. Significance was taken at p < 0.05.

**Results**

**Intrinsic Effects of CE.** CE as monotherapy had no measurable effects on parkinsonian monkeys with severe motor impairment. Motor disability scores remained unchanged during the 2 h after administration of every CE dose. Scores averaged 15.5 ± 2.5, 15.2 ± 2, 15.1 ± 2, 15.1 ± 2, and 15.6 ± 2.2 after CE vehicle, 0.03, 0.1, 0.3, and 1 mg/kg, respectively (p > 0.05). Although monkeys had a tendency to relax, motor disability was not worsened (unchanged scores in the scale). Monkeys did not exhibit retching, vomiting, or changes in social interaction. The highest doses of CE produced slight somnolence in two animals. This effect was observed irregularly, and it did not affect the interaction of the animal with the examiner.

CE was also tested as monotherapy in moderately parkinsonian monkeys during the study of comparison with L-DOPA (crossover). CE per se did not have motor effects in this group of monkeys either. Since in this study CE monotherapy was also evaluated after monkeys received L-DOPA tests, results show that monotherapy with CE had no effect in treatment-naive or L-DOPA-primed monkeys, i.e., responses to CE did not change between the groups (Table 3). Monkeys that received CE treatment after developing antiparkinsonian responses to L-DOPA had no antiparkinsonian response to CE, just as monkeys that received CE treatment initially. L-DOPA and CE responses were clearly different (p < 0.001; Table 3). Alternatively, the antiparkinsonian effects of L-DOPA before and after CE administration were similar in all animals. Therefore, CE did not potentiate initial responses to L-DOPA. Nor did it modify the course of development and stabilization of L-DOPA responses. Together, these results indicated that CE had no intrinsic effects on motor behavior of parkinsonian monkeys.

**Effects of CE on Antiparkinsonian Action of L-DOPA.** Coadministration of CE with L-DOPA resulted in enhanced antiparkinsonian effects. Responses to the suboptimal dose of levodopa were prolonged by the addition of CE in parkinsonian monkeys with different degrees of disability. In the severely parkinsonian group, a significant effect (CE + LD-low) was found on motor disability scores accumulated over the whole antiparkinsonian response, total scores (Fig. 2A). These effects were dose-dependent, and they were clearly manifested at the two higher doses, 0.3 and 1 mg/kg (p < 0.05 compared with baseline response of L-DOPA low dose, CE vehicle + LD-low). The reduction of total disability scores by adding CE derived from extended duration of low scores as shown in Fig. 2B (p < 0.01 for differences of scores at late individual intervals). Prolongation of L-DOPA responses varied among animals from 20 to 40 min after coadministration of CE, and this time represents 30% increase of response duration for L-DOPA low doses, which average 100 min. Coadministration of CE with L-DOPA optimal doses resulted in slighter effects in severely parkinsonian monkeys, suggesting that a maximal effect was reached (difference of scores at individual time points did not attain significance). The minimal scores obtained at the peak of L-DOPA effects (LD-low and LD-high) did not significantly change with the addition of any dose of CE.

In the moderately parkinsonian group, a significant effect of CE in combination with LD-low was also found on total motor disability scores accumulated during the antiparkinsonian response (p < 0.01; Fig. 2A). The addition of CE at the

![Fig. 2. Effects of coadministration of CE with L-DOPA on motor disability scores in severely parkinsonian monkeys. A, total motor disability scores. Each bar represents the effect of each treatment. CE vehicle (V) + LD-low, 0.1 mg/kg CE + LD-low, 0.3 mg/kg CE + LD-low, and 1 mg/kg CE + LD-low; and the same CE combinations with LD-high. Values are the average of the sum of each interval total scores during the whole antiparkinsonian response (from 30 min after treatment to the last interval of scores 50% or lower than baseline) from all monkeys. *, p < 0.05 versus CE vehicle + same dose of L-DOPA, B, time course of responses. Each curve represents the effects of each treatment combination of CE and LD-low, as described in A. Values are the average of total motor disability scores for individual intervals from all monkeys. "Off" represents the baseline score taken just before drug injections (time 0), and after injection, scoring starts at 30 min and follows thereafter every 20 min. **, p < 0.01 for differences in same time point between 0.3 mg/kg CE + LD-low or 1 mg/kg CE + LD-low and CE vehicle and LD-low (ANOVA for repeated measures; n = 3). Data points are mean ± S.E.M.
highest dose (1 mg/kg) had a tendency to reduce motor disability to lower scores at the peak effect, and it extended the reversal of parkinsonism from 80 to 110 min (Fig. 3B). L-DOPA high doses produced a marked response in this group that remained unchanged by the addition of CE.

On average from all parkinsonian monkeys, a substantial difference between treatments is demonstrated at 110 min from the injections, and it is maintained at 130 min. Figure 4 shows changes of response duration with coadministration of CE and LD-low in every monkey. Scores are also lower during the peak effect with the combination of 1 mg/kg CE and LD-low, although these differences were inconsistent.

**Effects of CE on L-DOPA-Induced Dyskinesias.** CE had no effects on L-DOPA-induced dyskinesias. Severely parkinsonian monkeys had stable dyskinetic responses to L-DOPA. Dyskinesias were typically peak-dose and of choreic type, although dystonic dyskinesias were also seen with the tested doses of L-DOPA. Mostly neck and trunk dystonia together with choreic movements of the limbs was present with the higher doses of L-DOPA. Dystonia was seen in the legs less frequently. Figure 5 shows scores of dyskinesias in each interval for all treatment combinations of CE and L-DOPA. A slight difference (nonsignificant) in values is present in the late intervals; thus, this effect is probably related to the prolongation of levodopa action by the addition of CE. The peak values of dyskinesias were not changed with the addition of CE. CE had no significant effect on individual scores of dyskinesias at any given time point (Fig. 5, A and B).

**Plasma Levels of L-DOPA.** CE had no effects on L-DOPA pharmacokinetics. Following systemic administration of L-DOPA plus benserazide at the suboptimal doses, L-DOPA concentration in plasma increased steadily until reaching its peak at 45 min, and then it gradually decreased during the remaining 100 min of behavioral effects (Fig. 6). Curves of plasma levels of L-DOPA were similar following coadministration of CE vehicle and the highest CE dose (1 mg/kg) that prolonged L-DOPA responses. No significant differences in plasma concentrations of L-DOPA were found in samples taken at any time during repeated determinations in two monkeys (Fig. 6). In fact, plasma levels of L-DOPA were slightly lower after administration of CE than after vehicle. Thus, the effects of CE on prolonging the response to L-DOPA were not due to prolongation of L-DOPA plasma concentration.

**Discussion**

Results of this study demonstrate that CE potentiates the antiparkinsonian action of levodopa in MPTP-treated rhesus monkeys. CE, in a dose-dependent manner, augmented L-DOPA responses by lowering scores of motor disability at the peak effect, and more markedly, by prolonging effects by 30% of the usual duration after a suboptimal dose of L-DOPA. This prolongation of L-DOPA response was not related to pharma-
cokinetin interactions, because plasma levels of L-DOPA were not affected by CE coadministration. CE effects are, thus, mediated by its central action at the CB1 receptors where this drug exerts full antagonism.

These data originally show that blockade of CB1 receptors augments the antiparkinsonian effects of L-DOPA in the rhesus monkey. The study also shows that CB1 receptor antagonism has 1) no intrinsic antiparkinsonian effects and 2) no antidyskinetic effects. Previous studies of cannabinoid agonists and antagonists have shown conflicting results where, for example, antagonists were found to be effective as monotherapy and to have antidyskinetic effects (Fernandez- Espejo et al., 2005; van der Stelt et al., 2005). Because we used MPTP-treated macaques that closely mimic motor behavior and basal ganglia anatomy of humans, and we used the inclusive experimental design from monotherapy to multiple aspects of levodopa coadministration, this study provides critical data to clarify the potential role of CB1 antagonists in the therapy of PD. Most previous work in primates derives from trials with the CB1 antagonist rimonabant (SR 141716A) in marmosets. Despite a homologous neuroanatomy (Hardman et al., 2002), definite differences between these nonhuman primate species are evidenced by the sensitivity to MPTP and the responsiveness to L-DOPA. In marmosets, a stable parkinsonism is rapidly produced (5 days), and dyskinesias are induced after short drug exposure (10 days) (Pearce et al., 1995), as opposed to several months of both MPTP and L-DOPA treatments required in macaques. In addition, different motor behavior of marmosets (high baseline activity) is commonly assessed with locomotor activity and range of movement. Results from these measurements are not equivalent to our scoring system using standardized scales that were designed to quantify specific parameters of parkinsonian disability, such as tremor, posture, and stability. Therefore, this study presents unequivocal data that demonstrate potentiation of L-DOPA antiparkinsonian effects by CB1 antagonists. These drugs have barely been tested in parkinsonian patients by limited trials of a single challenge with a single dose (Mesnage et al., 2004). Our results support a thorough clinical evaluation of CB1 antagonists.

CE had antiparkinsonian effects in our two groups of monkeys that differed not only in their motor impairment but also in their responsiveness to L-DOPA. High sensitivity in severe conditions leads to a similar high effect close to a “ceiling” effect with different doses of L-DOPA (“all or none” response) (Nutt and Holford, 1996). Severely parkinsonian monkeys also have motor fluctuations typically characterized by shortened responses, and they may have a reduced capability to adjust the response duration to different doses (Nutt, 2001). Thus, motor responses to dopamine in the advanced stages of the disease involve altered dopamine-mediated mechanisms, and results of CE in both conditions of parkinsonism suggest a cannabinoid mechanism that is independent of direct interaction with a certain dopamine signaling.

Lack of intrinsic effects of CB1 antagonists was also found in cynomolgus monkeys with rimonabant (Meschler et al., 2001). Here, CE was tested in monkeys naive of antiparkinsonian treatment to reveal slighter motor effects that are overshadowed after development of full responses to dopamine stimulation in conditions of marked motor impairment.
In summary, results of this study indicate that the specific effects of CB1 receptor blockade by systemic administration of selective antagonists consist of enhancement of antiparkinsonian responses to dopaminergic stimulation. These effects are expressed in moderately as well as severely parkinsonian rhesus monkeys that have altered responses to levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. Eur J Neurosci 18:1607–1614.

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Di Marzo V, Hill MP, Bisogno T, Crossman AR, and Brotchie JM (2000) Enhanced antiparkinsonian effects depend on modulation of dopamine responses. Lack of antidysskinetic effects of CE may be in line with the opposite effects of the cannabinoid agonist nabilone (Sieradzan et al., 2001; Fox et al., 2002). It remains unclear where cannabinoid antagonists and agonists mediate these opposing behavioral effects among the various locations of CB1 receptors in basal ganglia. The status of endocannabinoid transmission after dopamine denervation remains controversial. Changes of striatal endocannabinoids have been reported in opposite directions (Gubellini et al., 2002; Ferrer et al., 2003). In addition, CB1 receptor binding and mRNA were found to increase in striatum and other basal ganglia regions (Mailleux and Vanderhaeghen, 1993; Lastres-Becker et al., 2001). However, the precise function of this system through presynaptic and postsynaptic receptors remains obscure. CB1 receptors have modulatory effects on GABAergic, glutamatergic, and other activities of transmitters that may lead to varied interaction with dopamine responses (Di Marzo et al., 1998; Gubellini et al., 2002). As a result, CB1 antagonists have been proposed to reduce bradykinesia—antiparkinsonian action (Romero et al., 2000)—but also L-DOPA-induced dyskinesias—antidysskinetic action (Brotchie, 1998). In contrast, the activation of striatal CB1 receptors may produce motor effects by recognized mechanisms, such as the reduction of glutamate release (Gerdeon and Lovinger, 2001; Gubellini et al., 2002) and the expression of long-term depression (Gerdeon et al., 2002; Kreitzer and Malenka, 2007). In line with our results, Gubbrdida et al. (1999) have shown reciprocal interactions between endocannabinoid and dopaminergic systems in normal conditions where CB1 blockade facilitates dopaminergic effects. Some authors (e.g., Sieradzan et al., 2001) have proposed that the effects of cannabinoid antagonists are mediated by increase of GABA reuptake in GPe through presynaptic CB1 receptors (Manef et al., 1996). However, the facts that CB1 receptor activation also reduces GABA release and has important actions in striatum and other regions (Herkenham et al., 1990) rather suggest the interaction of various mechanisms to produce the observed behavioral effects following systemic administration of CB1 antagonists. Data presented here do not address the mechanisms of action of CE, for which behavioral effects of intracerebral drug injections targeting regions of basal ganglia need to be tested.

In summary, results of this study indicate that the specific effects of CB1 receptor blockade by systemic administration of selective antagonists consist of enhancement of antiparkinsonian responses to dopaminergic stimulation. These effects are expressed in moderately as well as severely parkinsonian rhesus monkeys that have altered responses to dopaminergic drugs. Therefore, selective CB1 receptor antagonists may prove useful as an adjuvant to dopamine replacement therapy in patients with various disease stages. Furthermore, combined treatment with cannabinoid antagonists may help adjust L-DOPA doses to delay or reduce disabling motor complications.

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