Influence of Chronic Bromocriptine and Levodopa Administration on Cerebral Type 1 Cannabinoid Receptor Binding

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ABSTRACT Objectives: The endocannabinoid system is an important modulatory system in the brain. Complex interactions with brain dopaminergic circuits have been demonstrated. The aim of this study was to investigate the in vivo effect of the commonly used antiparkinsonian drugs, levodopa (L-DOPA) and bromocriptine, on type 1 cannabinoid (CB1) receptors, using the PET radioligand [^{18}F]MK-9470. Experimental approach: Seventeen female Wistar rats were studied at baseline and after chronic exposure to either L-DOPA (6 mg/kg/day with 1.5 mg/kg/day carbidopa; $n = 6$), bromocriptine (4 mg/kg/day; $n = 5$), or saline ($n = 6$). [^{18}F]MK-9470 binding was assessed in vivo using small animal PET imaging. [^{18}F]MK-9470 parametric images were generated, anatomically standardized to Paxinos space and analyzed by voxel-based statistical parametric mapping (SPM2) and a predefined volume-of-interest (VOI) approach. Results: In a 2×2 analysis design (condition vs. treatment), no significant changes in absolute or relative [^{18}F]MK-9470 binding were present upon chronic exposure to L-DOPA or bromocriptine as compared to saline treatment. The post hoc comparison of chronic scans to baseline within each treatment modality showed regional increases in relative [^{18}F]MK-9470 binding in the thalamus (peak average value +6.3%) and in the sensorimotor cortex and hippocampus (peak average value +10.2%) after bromocriptine exposure, while no changes were found for L-DOPA. Conclusion: Chronic administration of L-DOPA and bromocriptine at the applied doses does not produce major cerebral changes in in vivo cannabinoid CB1 receptor binding of [^{18}F]MK-9470 in the rat brain. These results also suggest that similar chronic L-DOPA and bromocriptine usage is unlikely to interfere with human PET imaging in healthy conditions using this radioligand. **Synapse 64:617–623, 2010.** ©2010 Wiley-Liss, Inc.

INTRODUCTION

The endogenous cannabinoid system (ECS) consists of a family of naturally occurring lipids, the endocannabinoids, of which anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the best-characterized substances, of transport and degradation proteins, and of cannabinoid receptors (Di Marzo et al., 1998). Type 1 cannabinoid (CB1) receptors are abundantly expressed in all brain areas, especially those involved in the control of motor function, such as the basal ganglia, cerebellum, and sensorimotor cortex (Herkenham et al., 1991). Acting through the activation of G_i proteins, cannabinoid CB1 receptor stimulation is thought to modulate neuronal firing and neurotransmitter release in a dynamic, activity-dependent manner (Goutopoulos and Makriyannis, 2002).

In particular, the ECS acts as a modulator of dopaminergic activity in the basal ganglia through complex mechanisms (Ferrer et al., 2003). It has been shown that exogenous cannabinoids excite dopaminergic neurons in the substantia nigra (SN) of rats

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(French et al., 1997). Chronic treatment with dopamine D2 receptor antagonists upregulates cannabinoid CB1 receptor expression in the rat striatum and, conversely, injection of a cannabinoid receptor agonist into the basal ganglia counteracts the motor response of locally administered dopamine D2 receptor agonists (Jarrahian et al., 2004). Stimulation of dopamine D2 receptors leads to elevations in extracellular concentrations of AEA, but not of 2-AG, in the rat dorsal striatum (Giuffrida et al., 1999), while selective cannabinoid CB1 receptor antagonists enhance the antiparkinsonian action of dopaminomimetics in MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine)-treated rhesus monkeys (Cao et al., 2007).

Pharmacological agents which modify brain dopaminergic activity are standard therapy in movement disorders. Levodopa (L-DOPA) and bromocriptine are commonly used agents for the initial treatment of Parkinson's disease (PD) (Rao et al., 2006). L-DOPA is used as a prodrug to counteract the striatal dopamine deficiency caused by the massive degeneration of dopaminergic neurons in the SN pars compacta (Cotzias, 1968), while bromocriptine selectively stimulates dopamine D2 receptors (Calne et al., 1974). Although their mechanism of action on the dopaminergic system is well understood and the side-effects of long-term treatment of L-DOPA are characterized (Zesiewicz et al., 2007), their interaction with the ECS has not been investigated by in vivo imaging.

Positron emission tomography (PET) is a quantitative imaging technique which allows direct in vivo visualization of receptor binding and therefore also of interactions of administered drugs on receptor systems. In this study, we have investigated quantitatively the in vivo chronic effect of L-DOPA and bromocriptine on the cannabinoid CB1 receptor using small animal PET imaging and the high-affinity and subtype-selective PET radioligand [^{18}F]MK-9470 (*N*-[2-(3-cyano-phenyl)-3-(4-(2-[^{18}F]fluorethoxy)phenyl)-1-methylpropyl]-2-(5-methyl-2-pyridyloxy)-2-methyl proponamide) (Burns et al., 2007). The primary objective was to evaluate potential direct pharmacological interactions between L-DOPA/bromocriptine and cannabinoid CB1 receptor binding from a pathophysiological viewpoint. As secondary objective, the extent of possible confounding effect of chronic L-DOPA/bromocriptine administration on the in vivo cannabinoid CB1 receptor binding of [^{18}F]MK-9470 was assessed to facilitate and potentially correct human imaging studies.

MATERIALS AND METHODS

Direct binding affinity of L-DOPA and bromocriptine at the cannabinoid CB1 receptor was assessed using a standard competitive binding assay as previously described (Govaerts et al., 2004). Binding assays were conducted on membrane preparations from human cannabinoid CB1 receptor transfected Chinese hamster ovarian cells (CHO-CB1) incubated with 1 nM [^{3}H]-SR141716A (Amersham, Roosendaal, The Netherlands) and appropriate concentrations of competition ligand. Nonspecific binding was determined in the presence of 10 μM of the potent cannabinoid agonist HU 210 ((+)-1,1-dimethylheptyl analog of 7-hydroxy-delta-6-tetrahydrocannabinol; Tocris Cookson, Bristol, UK). It was assumed that direct binding affinity of both drugs at the human cannabinoid CB1 receptor is similar to the affinity at the rat cannabinoid CB1 receptor. Human and rat sequences are 90% identical in terms of nucleotides and 98% in terms of amino acids (Gerard et al., 1990).

Imaging experiments were conducted on 17 female Wistar rats (body weight range at the start of the experiment 204–282 g). All animals were housed three to a cage under controlled conditions of temperature, humidity, and 12-h light/dark cycle. Food and water were given ad libitum. The research protocol was approved by the local Animal Ethics Committee and was according to European Ethics Committee guidelines (decree 86/609/EEC).

The precursor for [^{18}F]MK-9470 was obtained from Merck Research Laboratories (MRL, West Point, USA). Radiolabeling was performed on-site by alkylation of the precursor with 2-[^{18}F]fluoroethylbromide. Tracer preparation and characteristics have been described elsewhere (Burns et al., 2007).

All chemicals were purchased from Sigma-Aldrich, Bornem, Belgium. L-DOPA (L-3,4-dihydroxyphenylalanine methyl ester) and the peripheral DOPA-decarboxylase inhibitor carbidopa were dissolved in a small amount of 0.2 N HCl and 0.2% (w/v) ascorbic acid (final concentration ascorbic acid: 0.02%). Solutions were titrated to pH 5.0–5.5 with 0.5 N NaOH. Compounds were then filtrated (filter pore size: 0.22 μm) and lyophilized for storage. The appropriate final concentration was obtained daily by adding distilled water. Bromocriptine (2-bromo-α-ergocryptine methanesulfonate salt) was suspended daily in 1% Tween 80-saline solution to achieve a final concentration of 1 mg/0.5 ml.

[^{18}F]MK-9470 PET imaging was performed in baseline condition and chronic exposure to L-DOPA (1 week of 6 mg/kg/day with 1.5 mg/kg/day intraperitoneal (IP) carbidopa administration; $n = 6$), bromocriptine (1 week of 4 mg/kg/day IP; $n = 5$), or saline (0.9% NaCl in 0.5 ml, 2 weeks IP; $n = 6$). The saline-treated rat data were used from similar pharmacological interaction studies where longer chronic exposures were used. The last dose of chronic treatment was administered at least 30 min before the small animal PET scan for L-DOPA, bromocriptine, and saline-treated animals. The applied dose for L-DOPA was obtained from typical therapeutic doses used in

Parkinsonian patients with relatively short disease duration and no dyskinesia (Martinelli et al., 2003). The L-DOPA dose and administration regimen chosen is within the range required to also ensure no abnormal involuntary movements in rodents (Lindgren et al., 2007; Morgese et al., 2007). The dose and administration frequency of bromocriptine produce in rats the same overall amount of motor activation as the dose of L-DOPA (Lindgren et al., 2007; Lundblad et al., 2002).

Prior to small animal PET imaging, rats were fasted overnight and anesthetized using an IP injection of 50 mg kg^{-1} sodium pentobarbital (Nembutal, Ceva Sante Animale, Brussels, Belgium). Small animal PET imaging was performed using a FOCUS 220 tomograph (Siemens/Concorde Microsystems, Knoxville, TN), which has a transaxial resolution of 1.35 mm full-width at half-maximum. Data were acquired in a 128 × 128 × 95 matrix with a pixel width of 0.475 mm and a slice thickness of 0.796 mm. Sixty-minute dynamic [^{18}F]MK-9470 acquisitions were started immediately after injection of ~18 MBq into the tail vein using an infusion needle set (Goffin et al., 2008).

Scans were reconstructed using filtered back projection and the last 20 min of each acquisition period were used for quantification purposes. Parametric images based on standardized uptake values (SUV) (activity concentration (MBq/ml) × body mass (g)/injected dose (MBq)) were generated as a measure for absolute [^{18}F]MK-9470 binding (Burns et al., 2007). No significant differences in weight or injected activity were present between baseline and chronic scans of drug- or saline-treated animals.

For each subject, parametric SUV images were spatially normalized to a stereotactic space based upon the rat brain Paxinos atlas (Casteels et al., 2006). We investigated both absolute (SUV) as well as relative cannabinoid CB1 receptor binding (SUV values normalized on whole-brain uptake).

Predefined volume-of-interest (VOI) and voxel-based statistical parametric mapping (SPM2) analyses were performed using a 2 × 2 design (condition vs. treatment). For maximal sensitivity, also a 2 × 1 design comparing chronic to baseline scans within each treatment modality was post hoc used. For VOI analysis, a predefined VOI map was loaded on all parametric images to permit calculation of average SUV's within each VOI (PMOD, version 2.65; PMOD, Zurich, Switzerland). The 2 × 2 analysis on VOI data was simplified by calculating the difference in SUV values between chronic and baseline in each group (saline, L-DOPA or bromocriptine treatment): $\text{difference}_{SUV} = (SUV_{chronic} - SUV_{baseline}) \times 2 / (SUV_{chronic} + SUV_{baseline})$. The difference_{SUV} values in each VOI of L-DOPA and bromocriptine-treated animals were then compared to the saline treatment by using a one-way analysis of variance (ANOVA) followed by two-sided Dunnett post hoc test (Statistica, version 8.0; Statsoft, Tulsa OK, USA). For the comparison of SUV values between chronic and baseline within each treatment modality, a paired T-test was used. All significances were accepted at the 95% probability level.

For SPM analysis, data were smoothed with an isotropic Gaussian kernel of 1.2 mm and analyzed both in a multisubject design using conditions (baseline vs. chronic) × subjects (L-DOPA or bromocriptine treatment vs. saline), as well as a single subject design using conditions only (baseline vs. chronic). Only significant clusters ($P < 0.05$, corrected for multiple comparisons) were retained, in combination with sufficient localizing power ($P_{height} < 0.005$, uncorrected for multiple comparisons), as described previously (Van Kuyck et al., 2007). The extent threshold k_E was 200 voxels (1.6 mm^3). For analysis of relative receptor binding, proportional scaling was used and an analysis threshold of 0.8 of the mean image intensity was applied.

RESULTS

In vitro direct binding affinity of L-DOPA and bromocriptine at the human cannabinoid CB1 receptor was negligible ($IC_{50} > 100$ μM). The percent displacement was 7.1% ± 8.1%, (mean ± SD) and 4.8% ± 17.0%, respectively at 10 and 100 μM for L-DOPA, while for bromocriptine 13.7% ± 2.1% and 18.9% ± 7.1% was found.

Mean cross-sectional small animal PET images of absolute [^{18}F]MK-9470 binding of the animals at baseline, and after chronic exposure to saline, L-DOPA, and bromocriptine are shown in Figure 1. The intersubject variability of absolute [^{18}F]MK-9470 binding ranged for all scans from 5 to 15%.

Chronic injections of saline did not produce significant changes in absolute or relative gray matter [^{18}F]MK-9470 binding (difference_{SUV}: + 4.2% ± 11.9% for whole-brain uptake).

In a 2 × 2 design, comparing L-DOPA and bromocriptine to saline-treated animals, we found no absolute or relative changes in [^{18}F]MK-9470 binding using both voxel-based and VOI-based analysis (Fig. 2).

Post hoc analysis, however, revealed regional changes in relative [^{18}F]MK-9470 binding upon chronic bromocriptine administration as compared to baseline, in the left thalamus (peak average value + 6.3%), and in a cluster covering the left sensorimotor cortex and left hippocampus (peak average value + 10.2%; Fig. 3). For L-DOPA, no significant changes were found. VOI-based analysis confirmed these findings. Detailed cluster peak locations and *P*-values for the comparison of chronic scan to baseline after bromocriptine administration are shown in Table I.

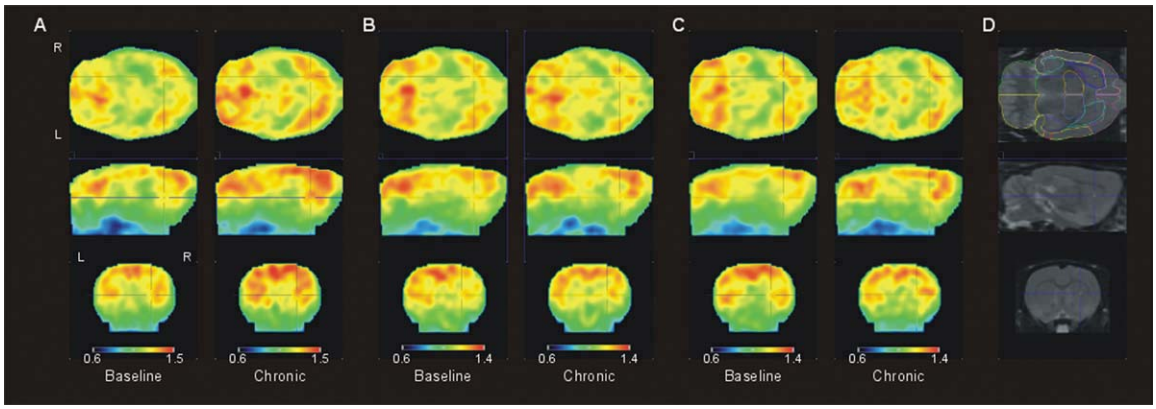

Fig. 1. Average crosssectional small animal PET images, coregistered to MRI (**D**), of absolute [^{18}F]MK-9470 binding at the midstriatal level after baseline, and after chronic exposure to saline (**A**), L-DOPA (**B**) and bromocriptine (**C**). The colored bars express SUV values. The intersection points of the three planes have been set to the mid-striatal level [i.e., (x, y, z) = (−2.9, −0.3, −5.6) Paxinos coordinates], which corresponds to the right hemisphere (L = left; R = right). The predefined volume-of-interest map is shown on the axial MRI image.

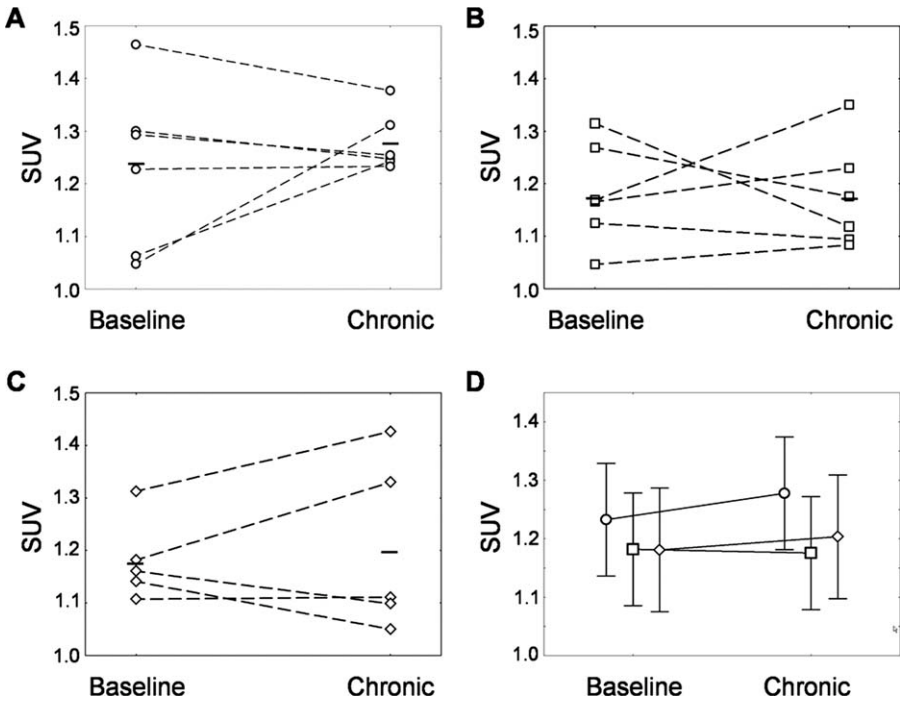

Fig. 2. Scatter plot of whole-brain gray matter standardized uptake values vs. condition for saline (**A**), L-DOPA (**B**) and bromocriptine (**C**) -treated rats respectively, as well as summarizing whisker plot (**D**). Horizontal lines indicate the mean SUV in each condition. Vertical bars denote 0.95 confidence intervals. ○, saline; □, L-DOPA; △, bromocriptine.

DISCUSSION

The ECS is an important neuromodulator, involved in the processing of cognitive, limbic, and motor information (Marsicano and Lutz, 2006). Many of these activities are also regulated by dopamine (Mehler-Wex et al., 2006; Nieoullon, 2002). The interaction between cannabinoid and dopaminergic systems in the brain has been demonstrated both in vitro and in vivo (French et al., 1997; Giuffrida et al., 1999; Jarrahian et al., 2004). In the present study, we investigated using small animal PET imaging whether the in vivo cannabinoid CB1 receptor binding of [^{18}F]MK-9470 is influenced by chronic exposure to L-DOPA and bromocriptine, two commonly used antiparkinsonian agents which modify brain dopaminergic activity. Since the intersubject variability of the imaging procedure is small (in the order of 10%), this technique allows measurements of small changes in abso-

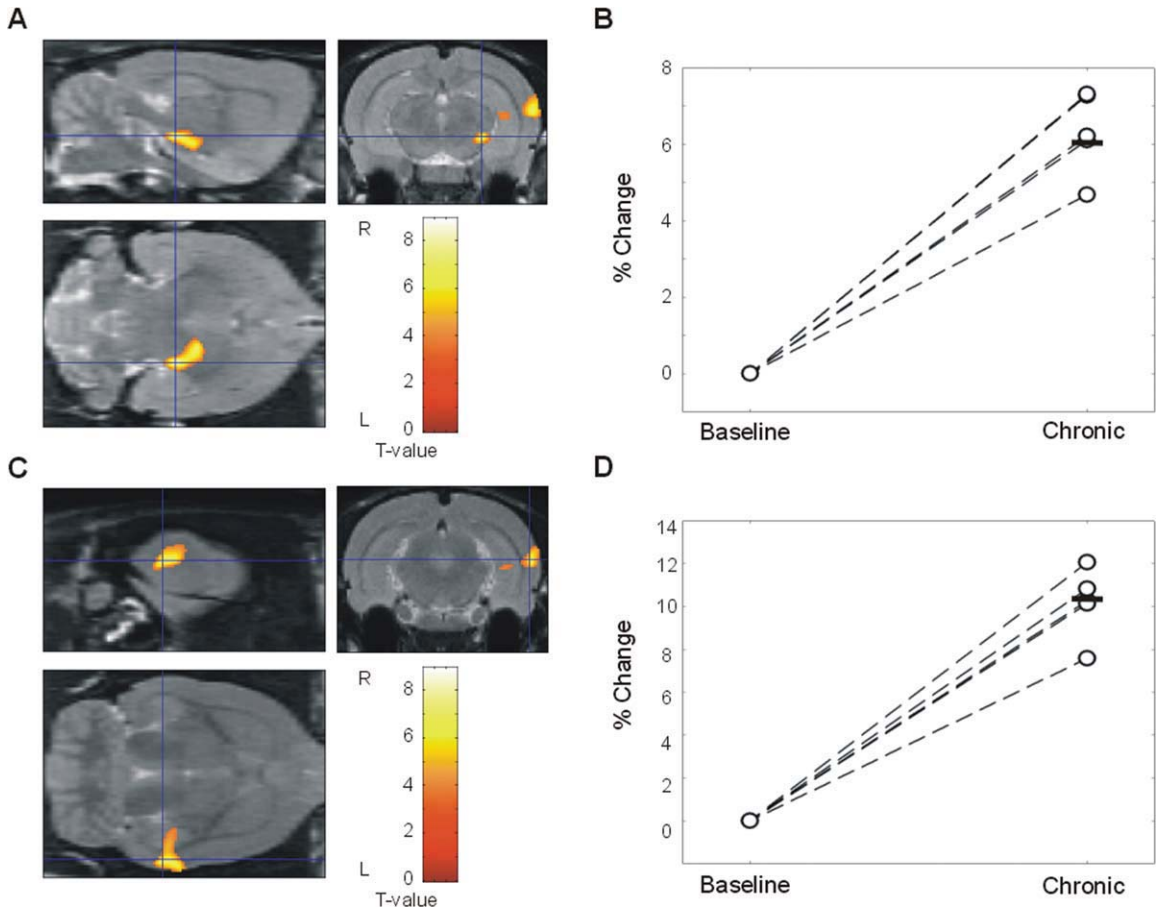

Fig. 3. Left: Statistical parametric maps of the difference in relative [^{18}F]MK-9470 binding in the thalamus (**A**) and sensorimotor cortex (**C**) of healthy rats after chronic exposure to bromocriptine as compared to baseline. Differences for the brain-regions have been color-coded and are superimposed on the MRI template in three orthogonal planes (L = left; R = right). The colored bars on the right express T-score levels. Right: The change in relative [^{18}F]MK-9470 binding expressed in terms of percentage at the maximal peak location in the thalamus (**B**) and the sensorimotor cortex (**D**). Mean values of increase are indicated by horizontal lines.

TABLE I. Peak locations for SPM2 comparison of baseline and chronic exposure to bromocriptine

Cluster-level		Voxel-level		Structure			
P_{corr}	k_E	T	P_{uncorr}	x	y	z	Name
<0.03	657	8.2	<0.001	3.0	−5.4	−6.8	Thalamus
		7.3	<0.001	2.0	−3.8	−7.0	
<0.04	638	6.5	<0.001	6.6	−6.4	−5.0	Sensorimotor Cortex
		6.5	<0.001	6.8	−5.4	−4.8	
		6.0	<0.001	7.2	−4.8	−4.4	
		4.3	0.001	4.9	−6.0	−5.5	Hippocampus

Bromocriptine: chronic > baseline (at $P_{height} < 0.005$ uncorrected).
P_{corr} at cluster level: the chance (P) of finding a cluster with this or a greater size (k_E), corrected for search volume; k_E = cluster extent; P_{uncorr} at voxel level: the chance (P) of finding (under the null hypothesis) a voxel with this or a greater height (T-statistic), uncorrected for search volume. x = lateral distance in mm from the midline (negative values to the right side); y = anteroposterior location relative to Bregma (negative values: posterior to Bregma); z = dorsoventral position (based upon the Paxinos stereotactic atlas).

lute cannabinoid CB1 binding. Modeling of tracer kinetics indicated that the area-under-the-curve macroparameter SUV is a good index of cannabinoid CB1 receptor binding at the measured scanning interval (Burns et al., 2007; Terry et al., 2009).

We found that there are no significant changes in cannabinoid CB1 receptor binding in the rat brain after chronic exposure to L-DOPA and bromocriptine when compared to the effect of saline treatment. Regionally detectable post hoc changes in relative [^{18}F]MK-9470 binding were present in the thalamus, sensorimotor cortex, and hippocampus after bromocriptine exposure only as compared to baseline, while no changes were found for saline or L-DOPA.

This finding in relative [^{18}F]MK-9470 binding in the thalamus, hippocampus, and sensorimotor cortex is unlikely the result of a direct interaction of bromocriptine with the cannabinoid CB1 receptor, as the in vitro binding affinity of the drug at the human receptor is negligible. We assume that the direct binding affinity at the rat cannabinoid CB1 receptor is similar to the reported direct binding affinity at the human receptor. Human and rat sequences are 90% identical in terms of nucleotides and 98% in terms of amino acids (Gerard et al., 1990), implying bromocriptine itself is unable to displace the high-affinity [^{18}F]MK-

9470 radioligand (rat IC_{50} = 0.9 nM) at the used dosage.

An indirect effect related to the ability of bromocriptine to modulate endocannabinoid levels in vivo cannot be excluded. It has been shown that acute activation of dopamine D2 receptors is accompanied by an eight-fold AEA release in the dorsal striatum of healthy rats (Giuffrida et al., 1999). Moreover, the response of cannabinoid receptors to agonist treatment is regionally dependent (Romero et al., 1995). Romero et al. detected in vitro increases in the receptor density of cannabinoid CB1 receptors primarily in the hippocampus after 5 days of daily exposure to AEA (3 mg kg^{-1}), while other brain regions remained unaffected (Romero et al., 1995).

An interaction between both neurotransmitter systems in the observed regions is anatomically plausible. Both cannabinoid CB1 receptors and the dopamine D2 receptors are colocalized in the molecular layer of the hippocampus (Egertova and Elphick, 2000) and in the anterior dorsal part of the thalamic nucleus (Moldrich and Wenger, 2000), as well as coupled to the same cellular effector system (Pertwee, 1999; Strange, 1993). Also, the somatosensory cortex expresses a high density of cannabinoid CB1 receptors particularly in deep layer projections to the dopamine-innervated striatum (Bodor et al., 2005).

However, modulation of endocannabinoid levels by L-DOPA has also been described. One single injection of high dose (50 mg kg^{-1}) L-DOPA selectively elevates AEA release by three to four times in the caudate-putamen, globus pallidus, and SN of normal rats, whereas no effect on 2-AG levels was observed (Ferrer et al., 2003). Chronic L-DOPA administration of the same dose for 11 days to intact animals produced only a modest, although not significant increase in AEA in the basal ganglia (Ferrer et al., 2003). We therefore hypothesize that at the applied therapeutic doses of L-DOPA and bromocriptine in this work, increases in endocannabinoid levels may occur, but that these are insufficient to cause major changes in cannabinoid CB1 receptor binding of [^{18}F]MK-9470, especially for L-DOPA.

Possible increases in regional cerebral blood flow (rCBF) caused by bromocriptine or L-DOPA could theoretically augment the tracer delivery to the brain and be responsible for higher tracer binding to the available cannabinoid CB1 receptors. Since we have observed only minimal effects of CBF changes on the SUV macroparameter using IV acetazolamide at concentrations that induce up to 70% CBF increases in the rat brain (Casteels et al., unpublished results), such possible CBF changes are unlikely to have confounded our results. Although data in the rat brain are lacking, the changes in CBF observed in human brain imaging studies after bromocriptine (Celsis et al., 1988) and L-DOPA (Kobari et al., 1995) exposure are small (in the order of 10–15%) and regionally invariant in the case of bromocriptine.

The administration regime of 1-week treatment is also unlikely to have biased our findings. Adaptive processes induced by dopaminergic treatment are well-described within this timeframe for other receptor systems, implying they are not too slow to show effects (De Sousa et al., 1997; Dirami and Cooke, 1998). However, direct correlational studies evaluating the dynamic pattern of changes in cannabinoid CB1 receptor binding after shorter/longer dopamine exposures further clarify this.

In humans, numerous studies additionally showed that disease states have profound influences on the neurotransmitter release following pharmacological interventions. While we demonstrate that L-DOPA and bromocriptine do not induce major changes in the cerebral [^{18}F]MK-9470 binding in healthy rats, this might not be true for experimental models of PD. There is evidence of increased striatal CB1 mRNA in 6-OHDA lesioned rats treated with L-DOPA, but not in nonlesioned animals (Zeng et al., 1999). However, nigrostriatal damage with 6-OHDA failed, unlike in intact rats, to elevate AEA release in the basal ganglia upon dopamine receptor activation (Ferrer et al., 2003). Other studies even reported no differences in the dopamine–endocannabinoid crosstalk between both conditions (Morgese et al., 2009).

In conclusion, 1-week chronic treatment of L-DOPA and bromocriptine at the applied doses does not induce major changes in the cerebral [^{18}F]MK-9470 binding in healthy rats. From a translational point of view, this may suggest that chronic L-DOPA and bromocriptine usage in the healthy situation is unlikely to interfere with in vivo cannabinoid CB1 receptor binding of [^{18}F]MK-9470. Whether the dopamine and cannabinoid system interact substantially different following chronic dopamine denervation needs further confirmation in direct PD studies.

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