Published in final edited form as:

Neurosci Res. 2012 March ; 72(3): 236–242. doi:10.1016/j.neures.2011.12.006.

The cannabinoid agonist WIN55212-2 decreases L-DOPA-induced PKA activation and dyskinetic behavior in 6-OHDA-treated rats

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Abstract

Chronic Levodopa (L-DOPA), the gold standard therapy for Parkinson's disease (PD), causes disabling motor complications (dyskinesias) that are associated with changes in the activity of striatal protein kinase A (PKA) and cAMP-regulated phosphoprotein of 32 KDa (DARPP-32). In this study, we showed that systemic administration of the cannabinoid agonist WIN55212-2 ameliorated L-DOPA-induced abnormal involuntary movements (AIMs) in the 6-OHDA rat model of PD and reversed L-DOPA-induced PKA hyperactivity via a CB₁-mediated mechanism. This effect was accompanied by increased phosphorylation of DARPP-32 at threonine 34, which was partially blocked by CB₁ antagonism. Striatal PKA activity was positively correlated with the severity of L-DOPA-induced axial and limb dyskinesias, suggesting a role for the cAMP/PKA signaling pathway in the expression of these motor disturbances.

Our results indicate that activation of CB₁ receptors, as well as reduction of striatal PKA hyperactivity, might be an effective strategy for the treatment of L-DOPA-induced dyskinesias.

Keywords

Parkinson's Disease; c-AMP-Regulated Phosphoprotein 32; Striatum; CB₁ receptor; cannabinoid; dopamine; dyskinesia

1. Introduction

Parkinson's Disease (PD) is a progressive neurodegenerative disorder characterized by severe motor impairment resulting from the loss of dopaminergic nigro-striatal neurons. The dopamine precursor 3,4-dihydroxyphenyl-L-alanine (L-DOPA) is the gold standard

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treatment to control PD symptoms in the initial phase of the disease. However, its therapeutic efficacy wanes with time, and development of “on”/“off” phenomena and disabling chorea-like involuntary movements, termed L-DOPA-induced dyskinesias (LID), have been reported in the majority of PD patients chronically treated with L-DOPA (Fahn 2006).

LID can be modeled via chronic administration of low doses of L-DOPA to rats with unilateral 6-hydroxydopamine (6-OHDA) lesions. This regimen produces increasingly severe abnormal involuntary movements (AIM) resembling the dyskinetic symptoms observed in PD patients (Lundblad et al. 2002). LID development and expression have been linked to several alterations in the basal ganglia circuitry, including increased responsiveness of striatal medium spiny neurons (MSN) to dopamine (Bezard et al. 2001; Olanow and Obeso 2000), pulsatile stimulation of dopamine receptors (Obeso et al. 2004), changes in L-DOPA and dopamine bioavailability (Carta et al. 2006; de la Fuente-Fernandez et al. 2004), dopamine release from serotonin terminals (Carta et al. 2008), increased dopamine D1 receptor-mediated signaling (Bezard et al. 2001) and maladaptive changes in synaptic plasticity (Cenci and Lundblad 2006; Picconi et al. 2003; Kreitzer and Malenka 2007). In addition, several studies have shown an association between the development of L-DOPA-induced abnormal motor responses and dysregulation of the cAMP/protein kinase A (PKA) signaling cascades, and of the dopamine- and cAMP-regulated phosphoprotein-32KDa (DARPP-32) (Oh et al. 1997; Picconi et al. 2003; Santini et al. 2007). DARPP-32 is highly expressed in MSN and its activity is regulated via two phosphorylation sites, threonine (Thr)-34 and Thr-75. PKA-mediated phosphorylation of Thr-34 converts DARPP-32 into an inhibitor of protein phosphatase 1 (PP1) (Hemmings et al. 1984), whereas phosphorylation at Thr-75 promotes inhibition of PKA activity (Bibb et al. 1999).

Experimental evidence points to the endocannabinoid system as a novel pharmacological target to treat LID, and either pharmacological blockade (Segovia et al. 2003; van der Stelt et al. 2005) or activation (Morgese et al. 2007; Papa 2008) of cannabinoid CB₁ receptors has been associated with beneficial effects on LID. CB₁ receptors are expressed in brain areas regulating motor function, including the basal ganglia, cerebellum and sensory-motor cortex (Mackie 2005), and activation of dopamine D2 receptors has been shown to promote endocannabinoid release (Giuffrida et al. 1999), which in turn decreases excitatory synaptic inputs in the striatum via activation of presynaptic CB₁ receptors located on glutamatergic terminals (Gerdeman and Lovinger 2001; Gubellini et al. 2002; Kreitzer and Malenka 2007; Tozzi et al. 2011). As the dopamine-dependent release of the endocannabinoid anandamide is disrupted following the 6-OHDA lesion of the nigrostriatal pathway (Morgese et al. 2007), it is likely that this deficiency might contribute to maladaptive synaptic plasticity within the striatum (Calabresi et al. 2007; Cenci and Lundblad 2006; Gubellini et al. 2002; Kreitzer and Malenka 2007), leading to the emergence of motor disturbances and dyskinesias. In keeping with this hypothesis, we previously showed that sub-chronic administration of the cannabinoid agonist WIN55212-2 (WIN), at doses that do not affect general locomotor activity nor dopamine-elicited motor responses, reduced L-DOPA-induced AIM in 6-OHDA-treated rats via a CB₁-dependent mechanism (Morgese et al. 2007; Morgese et al. 2009). As some of the psychomotor actions of cannabinoids are mediated through the PKA-dependent phosphorylation of DARPP-32 (Andersson et al. 2005), and increased DARPP-32 phosphorylation at Thr-34 has been associated with LID (Aubert et al. 2005; Santini et al. 2007), we investigated the molecular mechanisms associated with the antidyskinetic effects of WIN and assessed whether administration of L-DOPA, as monotherapy or in combination with WIN, affected PKA activity and/or DARPP-32 phosphorylation in the striatum of dyskinetic rats.

2. Material and Methods

2.1. Chemicals

Desipramine hydrochloride, L-DOPA methyl ester, 6-OHDA hydrochloride, benserazide hydrochloride, amphetamine and ketamine hydrochloride were purchased from Sigma Chemicals Co. (St. Louis, MO); WIN55,212-2 mesylate (WIN) was from Tocris Bioscience (Ellisville, MI) and AM251 from Cayman Chemical Co. (Ann Arbor, MI).

2.2. Animals

Male Wistar rats (225–250 g; Charles River Laboratories, Wilmington, MA) were housed on a 12 h light/dark cycle, at 22±1°C, with food and water available *ad libitum*. Animals were habituated to housing conditions for 1 week before the experiments. Animal care and experiments were conducted in accordance with the guidelines of the National Institutes of Health “Guide for Care and Use of Laboratory Animals” and approved by the Institutional Animal Care and Use Committees of the University of Texas Health Science Center at San Antonio. All efforts were made to minimize the number of animals used in the study and post-surgery distress.

2.3. 6-OHDA lesion

Rats were anesthetized with an injection of a cocktail (0.85 ml/kg, i.p.) containing ketamine (100 mg/ml), xylazine (100 mg/ml) and acepromazine (10 mg/ml) in saline. Dopamine-denervating lesions were carried out using a stereotaxic frame (Kopf Instruments, Tujunga, CA) via unilateral injection of 2 µl of 6-OHDA (4 µg/µl; flow rate 0.5 ml/min) dissolved in 0.1% ascorbate saline into the left medial forebrain bundle (in mm relative to Bregma: AP −4.3, ML +1.6, DV −8.3; tooth bar −2.4) (Paxinos and Watson 1998), using a 10 µl Hamilton microsyringe attached to a 30-gauge needle. The noradrenalin transporter inhibitor, desipramine (25 mg/kg, i.p.) was administered 30 min before 6-OHDA to protect noradrenergic neurons. Two weeks after surgery, all 6-OHDA-treated rats received an injection of *d*-amphetamine (2.5 mg/kg, i.p.) to assess the efficacy of the lesion by counting the number of amphetamine-induced rotations. Only animals showing more than 5 full ipsilateral rotations per min, corresponding to a dopamine depletion greater than 90% (Ferrer et al. 2003; Papa et al. 1994; Winkler et al. 2002), were included in the study. The extension of the striatal 6-OHDA lesion was confirmed post-mortem by measuring TH expression by western blot.

2.4. Behavioral studies

6-OHDA rats were treated chronically with a daily injection of L-DOPA (6 mg/kg, s.c.) plus benserazide (12.5 mg/kg, s.c.), or saline (controls) for 12 consecutive days to gradually induce abnormal involuntary movements (AIMs) as previously shown (Morgese et al. 2007). AIMs were grouped into three categories: (a) *axial*, i.e. twisting movements of the neck and upper body and dystonic posturing contralateral to the lesion; (b) *limb*, i.e. repetitive jerks and/or purposeless movement of the contralateral forelimb; (c) *oro-facial*, i.e. empty jaw movements and tongue protrusion (for details see (Morgese et al. 2007)). From day 9 to 12 of L-DOPA, animals received a daily injection of the cannabinoid agonist WIN (1 mg/kg, i.p.) or vehicle (5/5/90% of Tween80/PEG/saline, i.p.) 15 min before L-DOPA. On day 12, some animals were injected with the selective CB₁ antagonist AM251 (1mg/kg, i.p.) or vehicle (10/10/80% of Tween80/PEG/saline, i.p.) 15 min before WIN.

Axial, limb and oro-facial AIMs were monitored daily between 10:00 a.m. and 4:00 p.m. as previously described (Morgese et al. 2007). Briefly, animals were placed individually in a Plexiglas box and AIMs were scored by two observers blind to the treatments for 5 min intervals at 60 and 120 min after L-DOPA administration using a severity scale ranging

from 0 to 4 (for details see (Morgese et al. 2007)). Half-point scores were added to enhance the sensitivity of the rating scale. Data were presented as median of total AIM (axial+limb+orofacial) scores.

2.5. Western blotting

After the behavioral assessment, all animals were anesthetized with isoflurane (by inhalation) and their brains rapidly collected 60 or 120 min after the last injection of L-DOPA or vehicle, frozen in cold 2-methylbutane (-50°C), placed on an ice-cold stainless steel mould (Roboz; Rockville, MD), cut into 1 mm coronal slices using razor blades to dissect out the dorsal striatum ipsilateral to the 6-OHDA lesion. Striatal tissue was homogenized in ice-cold lysis buffer containing 50 mM Tris-HCl, 100 mM NaCl, 0.1% Triton X-100, 0.1% SDS, 1 mM Na_3VO_4 , 10 mM NaF, 1 mM EDTA, and 1% protease inhibitor cocktail (Sigma, St.Louis, MO) and centrifuged at 15,000 g for 30 min at 4°C . The protein concentration of the supernatants was determined using a Micro BCA Protein Assay kit (Thermo scientific, Rockford, IL) according to manufacturer instructions. Equal amounts of samples (20 μg of protein) were resolved by SDS-PAGE (10%), transferred onto PVDF membranes (0.2 μm), and incubated for 60 min in 5% fat-free milk in Tris Buffer Saline + 0.05% Tween-20 (TBS-T buffer) at 4°C . Membrane were successively incubated at 4°C overnight with the following primary antibodies (1:1000 dilution in TBS-T + 1% BSA): anti-DARPP-32 (Cell Signaling Technology, Danvers, MA), anti-phospho[Thr-34]-DARPP-32, anti-phospho[Thr-75]-DARPP-32 and anti- β -actin (Sigma Chemical co., St.Louis, MO). After 3×5 min washes in TBS-T, membranes were incubated with the appropriate secondary horseradish peroxidase-linked antibodies (1:2000) for 60 min at room temperature. Protein bands were visualized using an enhanced chemiluminescence detection kit (ECL, Amersham Pharmacia Biotech, Piscataway, NJ) and band immunoreactivity was analyzed by densitometry (relative optical density).

2.6. PKA activity

PKA activity was measured by ELISA in 96-well plates pre-coated with a synthetic peptide as substrate for PKA, using a commercially available Non-Radioactive PKA Kinase Activity Assay Kit (Assay Designs, Ann Arbor, MI) according to manufacturer's instructions. Briefly, dorsal striata were homogenized in lysis buffer (20 mM MOPS, 50 mM b-glycerolphosphate, 50 mM sodiumfluoride, 1 mM sodium vanadate, 5 mM EGTA, 2 mM EDTA, 1% NP_4O , 1 mM dithiothreitol, 1 mM benzamidine, 1 mM phenylmethanesulphonylfluoride, 10 mg/mL leupeptinandaprotinin) containing 1% protease inhibitor cocktail and centrifuged for 15 min at 15,000 g. The supernatant was collected and 2 μg of protein was incubated for 90 min at 30°C with or without (control) ATP (2 $\mu\text{g}/\text{ml}$ of Kinase Assay Dilution Buffer) to initiate the reaction, followed by 60 min incubation in rabbit polyclonal antibody specific for the phosphorylated substrate (1 mg/ml solution) at room temperature. HRP-conjugated anti-rabbit IgG was added as secondary antibody and allowed to react for 30 min at room temperature. Color development was carried out by adding 60 ml of TMB (tetramethylbenzidine) and stopping the reaction after 30 min with 20 ml of acid stop solution. Absorbance was read in a plate reader at 450 nm.

2.7. PP2A and PP2B activity

Phosphatase activity was measured using the non-radioactive Serine/Threonine (Ser/Thr) Phosphatase Assay System (Promega, Madison, WI) according to manufacturer's instructions. Briefly, striatal tissue (about 15 mg) was homogenated in Tris buffer (10 mM, pH 7.5) containing 1% protease inhibitor cocktail at $0-4^{\circ}\text{C}$ and centrifuged at 100,000 g at 4°C for 60 min. Supernatants were filtered through a Sephadex G-25 column to remove free phosphates, and samples were collected and incubated, with or without (control) substrate (100 mM Ser/Thr phosphopeptide), in 5X PPase-2A (250 mM imidazole pH 7.2, 1 mM

EGTA, 0.1% β -mercaptoethanol, 0.5 mg/ml BSA) or PPase-2B (250 mM imidazole pH 7.2, 1mM EGTA, 50 mM MgCl_2 , 5 mM NiCl_2 , 250 mg/ml calmodulin, 0.1% β -mercaptoethanol) reaction buffer for 15 min at 30°C. The reaction was stopped by adding a molybdate dye mixture and left at room temperature for 30 min to allow for color development. Optical density was measured in a plate reader at 600 nm.

2.8. Statistical analysis

AIM data were expressed as median scores and analyzed using the Kruskal-Wallis test followed by Dunn's multiple comparison test (for inter-group analysis) and the Friedman test followed by Dunn's multiple comparison test (for intra-group analysis). All other data were expressed as the mean $\pm$ S.E.M. and analyzed by ANOVA followed by Tukey's test for multiple comparisons. The correlation between PKA activity and the AIM scale (dyskinesia severity) was calculated using a nonparametric tie-corrected Spearman's rank correlation. The threshold for statistical significance was set at $p < 0.05$.

3. Results

6-OHDA-treated rats undergoing chronic L-DOPA administration (6 mg/kg, s.c., 1 injection/day for 12 days) showed increasingly severe total AIMS (axial + limb + oro-facial) over time, reaching a plateau around day 8 of L-DOPA (Fig. 1). No AIMS were observed in 6-OHDA-treated rats receiving vehicle (data not shown). Systemic WIN (1 mg/kg, i.p., 15 min before L-DOPA, day 9–11) significantly attenuated L-DOPA-induced AIMS at 60 (Fig. 1A) and 120 min (Fig. 1B) after L-DOPA injection on day 10 and 11, whereas no significant effect was observed on day 9 (Kruskal-Wallis followed by Dunn's Multiple comparison test, $**p < 0.01$; $*p < 0.05$). The beneficial effects of WIN were acutely reversed by the selective CB_1 receptor antagonist AM251 (1 mg/kg, i.p., 15 min before WIN on day 12 of L-DOPA) at both time points (Fig. 1). AM251 had no effect on AIMS when given alone on the last day of L-DOPA treatment (data not shown).

To investigate whether WIN affected the signaling pathways associated with dyskinetic behaviors, L-DOPA-treated 6-OHDA rats receiving WIN or vehicle (controls) were sacrificed immediately after the behavioral assessment (60 and 120 min after L-DOPA injection) to collect the dorsal striatum ipsilateral to the lesion and measure PKA and DARPP-32 expression and activity. As revealed by western blot analysis, chronic L-DOPA had no effect on PKA expression (Fig. 2A), but significantly elevated PKA activity in dyskinetic rats at 60 (table 1) and 120 min (Fig. 2B) after L-DOPA injection ($p < 0.05$ and $p < 0.01$, respectively).

ANOVA followed by Tukey's multiple comparison test). Sub-chronic administration of WIN (day 9–11 of L-DOPA) prevented L-DOPA-induced elevation of PKA activity at both time points (Table 1 and Fig. 2B), and the selective CB_1 antagonist AM251 (1 mg/kg, i.p., 15 min before WIN) reversed this effect (Fig. 2B) without affecting PKA expression (Fig. 2A). AM251 did not affect either PKA expression or activity when administered alone (data not shown). PKA activity at 120 min was positively correlated with total AIM scores (Spearman $r = 0.734$, $p = 0.045$), as well as with axial (Spearman $r = 0.827$, $p = 0.015$) and limb AIMS (Spearman $r = 0.728$, $p = 0.045$) (Fig. 3). By contrast, we found no significant correlation between PKA activity and oro-facial AIMS (Spearman $r = 0.621$, $p = 0.115$) (Fig. 3).

To assess whether the L-DOPA-induced PKA activity in dyskinetic rats was accompanied by changes of downstream DARPP-32 signaling, we measured striatal DARPP-32 expression and phosphorylation at thr-34 and thr-75 in 6-OHDA-treated animals receiving vehicle (controls), L-DOPA and L-DOPA+WIN (same treatment as above). ANOVA

revealed no significant effect of lesion, L-DOPA or WIN treatment on total DARPP-32 expression (Fig. 4A). Chronic L-DOPA had no significant effect on DARPP-32 phosphorylation at 60 min (Table 2), but it significantly increased p-Thr34-DARPP-32 at 120 min (Fig. 4B and Table 3). WIN application reversed the L-DOPA-induced phosphorylation of Thr34 and the CB₁ antagonist AM251 partially blocked WIN effect (Fig. 4B).

The WIN-induced reduction of p-Thr34 was not accompanied by changes in the expression or activity of striatal calcineurin (PP2B), a phosphatase implicated in the dephosphorylation of DARPP-32 at thr-34 (table 1). No changes in p-Thr75-DARPP-32 (Table 2,3 and Fig. 4C), as well as in the activity of PP2A (Table 1), a phosphatase implicated in the dephosphorylation of this site, were observed in all experimental groups.

4. Discussion

Our study showed that subchronic systemic administration of the cannabinoid agonist WIN ameliorated L-DOPA-induced AIMs in 6-OHDA-treated rats and reversed the concomitant activation of PKA via a CB₁-mediated mechanism. We also found a positive correlation between the L-DOPA-induced PKA activation and the severity of axial and limb, but not orofacial, AIMs.

These data confirm previous observations showing that stimulation of CB₁ receptors significantly reduces LID in 6-OHDA-treated rats (Ferrer et al. 2003; Morgese et al. 2007; Walsh et al. 2010) and Rhesus monkeys (Cao et al. 2007). We did not observe any changes in L-DOPA-induced AIMs after acute administration of AM251. Similarly, no anti-dyskinetic effects were observed by other groups, even after prolonged CB₁ antagonism (Cao et al., 2007; Walsh et al., 2010). By contrast, studies carried out in reserpine-treated rats (Segovia et al., 2003) and MPTP-treated marmosets (van der Stelt et al., 2005) have reached opposite conclusions, suggesting that blockade, rather than activation, of CB₁ receptors ameliorates LID. These discrepancies might be attributed to species-specific differences in the response to cannabinoid pharmacological challenges across different animal models of PD, and/or to the selection of behavioral measures (e.g., vertical motor activity, as in the study of Segovia et al.), which may not strictly model dyskinesias (Cenci et al. 2002). Nevertheless, our study cannot rule out that higher doses and/or chronic application of AM251 might produce different behavioral outcomes, likely due to aspecific drug effects and/or changes in synaptic plasticity following long-term blockade of endocannabinoid signaling.

Our observations confirm previous reports showing an association between LID and the persistent activation of the cAMP/PKA pathway in the striatum of dyskinetic animals (Aubert et al. 2005; Picconi et al. 2003; Santini et al. 2008; Tong et al. 2004), and support recent studies suggesting that attenuation or pharmacological blockade of PKA signaling represents an effective strategy to reduce AIM expression and development (Lebel et al. 2010; Santini et al. 2007). In keeping with these observations, PKA inhibition has been shown to prevent L-DOPA-induced cytoskeleton modifications, such as hyperphosphorylation of the microtubule-associated protein tau, which may contribute to long-lasting synaptic plasticity changes underlying striatal dysfunction and motor impairment (Cyr et al. 2003; Lebel et al. 2010). On the other hand, the lack of correlation between PKA activity and orofacial AIMs indicates that PKA may not play a significant role in the aberrant plasticity underlying this dyskinesia subtype.

The striatal content of PKA protein was not altered by our pharmacological manipulations, suggesting that the L-DOPA-induced activation of PKA was not due to over-expression of

the enzyme, but it likely resulted from stimulation of dopamine receptors. Indeed, in dyskinetic animals chronically treated with L-DOPA, activation of the striatal cAMP/PKA signaling pathway has been linked to dopamine D1 receptor activation, which is known to contribute to the development of aberrant motor responses (Oh et al. 1997) and to stimulate the PKA-catalyzed phosphorylation of DARPP-32 at Thr-34 (Fisone et al. 2007; Santini et al. 2010). On the other hand, the reduction of PKA activity observed after systemic administration of WIN may result from the direct activation of CB₁ receptors, which are negatively linked to adenylyl cyclase (Bidaut-Russell et al. 1990; Piomelli 2003) and co-localized on D1-expressing neurons (Hermann et al. 2002). The L-DOPA-induced activation of PKA and its reversal by WIN were accompanied by parallel changes in DARPP-32 phosphorylation at Thr-34. Indeed, 60 min after L-DOPA application, we observed a slight increase of striatal p-Thr34-DARPP-32 that reached statistical significance at 120 min and was reversed by WIN co-administration. Our findings confirm previous studies showing enhanced DARPP-32 phosphorylation at Thr-34 in the striatum of dyskinetic 6-OHDA-treated rats (Picconi et al. 2003), but differ from other observations showing that DARPP-32 activation (i.e. increased phosphorylation at Thr-34) is required for the expression of cannabinoid-mediated motor effects (Andersson et al. 2005). The reason for this discrepancy requires further investigations and may depend on different biochemical/functional aspects underlying the behaviors measured in our versus Andersson's study (dyskinesia and catalepsy, respectively), and/or species-specific differences between rats and mice. Interestingly, Polissidis et al. (2010), found that a dose of WIN identical to that used in our experiments (1 mg/kg, i.p.) induced opposite changes in striatal Thr-34 phosphorylation (decrease rather than increase) in different rat strains (Polissidis et al. 2010). In addition, the WIN-induced dephosphorylation of Thr34-DARPP-32 was only partially dependent on CB₁ receptor activation, as it was not fully reversed by the selective CB₁ antagonist AM251, even when given at a dose that fully blocked WIN anti-dyskinetic effects. Nevertheless, we cannot rule out that the full reversal of WIN effect by AM251 might have occurred at later time points that were not included in our experimental design, and/or might have been restricted to specific subpopulations of striatal neurons (i.e. striatopallidal neurons) (Andersson et al. 2005) or striatal sub-regions enriched in CB₁ receptors that could not be evidenced using western blot analysis of homogenates. This possibility is supported by the observation that the distribution of CB₁ receptors in rat striatum is not homogenous, but follows a lateral to medial density gradient (Tsou et al. 1998).

Calcineurin (PP2B) is one of the few phosphatases acting at serine/threonine residues (Sim et al. 2003; Winder and Sweatt 2001) and implicated in the dephosphorylation of Thr34-DARPP-32 (Heifets et al. 2008). In the rat peripheral nervous system, WIN application has been shown to decrease PP2B activity via a CB₁-independent mechanism (Patwardhan et al. 2006). In our study, however, striatal PP2B activity was not affected by WIN administration (nor by the 6-OHDA lesion or chronic L-DOPA), suggesting that WIN promoted Thr34-DARPP-32 dephosphorylation via an alternative and unidentified mechanism.

As previously shown (Andersson et al. 2005), the L-DOPA-induced elevation of PKA activity was not accompanied by dephosphorylation of DARPP-32 at Thr-75, a site that, upon phosphorylation, converts DARPP-32 into a potent PKA inhibitor (Bibb et al. 1999; Nishi et al. 2002). In agreement with this observation, we found no changes in the activity of the striatal phosphatase PP2A, which has been shown to dephosphorylate this site (Ahn et al. 2007; Fernandez et al. 2006).

WIN may exert its antidyskinetic action by modulating striatal neurotransmission via a PKA-dependent mechanism affecting the exocytosis of synaptic vesicles (Seino and Shibasaki 2005) and/or endocannabinoid-mediated plasticity at excitatory and inhibitory synapses (Chevalleyre et al. 2007; Heifets et al. 2008; Mato et al. 2008; Pan et al. 2008). In

particular, recent studies have shown that endocannabinoids released from striatal medium spiny neurons can retrogradely activate presynaptic CB₁ receptors to depress synaptic transmission at excitatory cortico-striatal terminals and facilitate striatal long-term depression (LTD) (Ade and Lovinger 2007). Furthermore, stimulation of dopamine D2 receptors, which leads to anandamide release in the striatum (Giuffrida et al. 1999), can facilitate endocannabinoid-mediated striatal LTD (Kreitzer and Malenka 2007) by enhancing CB₁-mediated downstream inhibitory effects on cAMP/PKA signaling in GABAergic neurons (Pan et al. 2008). Therefore, given the deficiency in anandamide production observed in 6-OHDA-treated rats upon chronic L-DOPA administration (Kreitzer and Malenka 2007; Morgese et al. 2007), the consequent decrease in anandamide-mediated CB₁ stimulation may lead to alterations of striatal LTD, which in turn may contribute to LID development. In keeping with this hypothesis, Haj-Dahmane et al. (2010) have recently shown that inhibition of presynaptic PKA activity is required for endocannabinoid-mediated LTD in rat midbrain dopamine neurons (Haj-Dahmane and Shen 2010). In this context, the activation of CB₁ receptors by exogenous application of WIN could rebalance this maladaptive plasticity by inhibiting L-DOPA-induced PKA hyperactivity and consequently reduce glutamatergic input at striatal synapses.

In conclusion, this study further supports the hypothesis that the cAMP/PKA signaling pathway plays a critical role in the expression of L-DOPA-induced AIMs in 6-OHDA-treated rats and suggests that systemic administration of cannabinoid agonists may provide therapeutic relief by reversing the aberrant L-DOPA-evoked downstream signaling that may lead to long-term maladaptive changes in striatal plasticity.

Acknowledgments

We thank Drs. Alexandre Seillier and Julien Matricon for the critical reading of the manuscript. This study was supported by the National Institute of Health, NS050401-07 (to A.G.) and 1F31NS073411-01 (to A.M)

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Highlights

- Activation of cannabinoid CB1 receptors ameliorates L-DOPA-induced dyskinetic behavior in rats
- Cannabinoid agonism reduces the cAMP/PKA pathway hyperactivity associated with dyskinesia
- Cannabinoid drugs are promising therapeutics for the treatment of Parkinson's disease

Figure 1.

Time course of the effects of systemic administration of L-DOPA (filled squares, 6 mg/kg, 1 injection per day, $n=8$) in rats with unilateral 6-OHDA lesions on total abnormal involuntary movements (total AIMs, axial + limb + orofacial) measured at 60 (A) and 120 (B) min after L-DOPA injection. A different group of rats ($n=8$), chronically treated with L-DOPA as reported above, received the cannabinoid agonist WIN (diamonds, 1 mg/kg, i.p., from day 9 to day 12, 15 min before L-DOPA) and the CB₁ antagonist AM251 (inverted triangle, 1 mg/kg, i.p. 15 min before WIN) on day 12 of L-DOPA treatment. * $p<0.05$, ** $p<0.01$ compared to rats treated with L-DOPA + vehicle (Kruskal–Wallis followed by Dunn’s multiple comparison test). AM251 effect was analyzed using the Friedman test followed by the Dunn’s multiple comparison test. Values represent median score. The interquartile ranges have been omitted for clarity. Arrows indicate days of WIN administration.

Figure 2.

Expression of PKA levels (A) and activity (B) in the striatum ipsilateral to the 6-OHDA lesion of dyskinetic rats (n= 6–8 per group) undergoing chronic L-DOPA treatment for 12 days (6 mg/kg, 1 injection per day) and sacrificed 120 min after the last injection of L-DOPA. Data are expressed as percentage of controls (dash line). Empty bars, L-DOPA +vehicle; filled bars, L-DOPA+WIN; gray bars, L-DOPA+WIN+AM251. **Insets show representative SDS-PAGE gels. S, saline; LD, L-DOPA; W, WIN55212-2; AM, AM251.** **p<0.01: compared to vehicle-treated controls; #p<0.05: compared to L-DOPA+vehicle; ^P<0.05: compared to L-DOPA+WIN. ANOVA followed by Tukey's post hoc.

Figure 3. Correlations between PKA activity (expressed as % of control) and Total (A), Axial (B), Limb (C) and Oral (D) AIMs in the 6-OHDA-treated rats ($n = 8$ per group) undergoing chronic L-DOPA treatment for 12 days (6 mg/kg, 1 injection per day) and sacrificed 120 min after the last injection of L-DOPA.

Figure 4.

Expression of Total DARPP-32 (A), p-Thr34-DARPP-32 (B) and p-Thr75-DARPP-32 (C) in the striatum ipsilateral to the 6-OHDA lesion of dyskinetic rats ($n=6-8$ per group) undergoing chronic L-DOPA treatment for 12 days (6 mg/kg, 1 injection per day) and sacrificed 120 min after the last injection of L-DOPA. Data are expressed as percentage of saline-treated controls (dash line). Empty bar, L-DOPA+vehicle; filled bars, L-DOPA+WIN; gray bars, L-DOPA+WIN+AM251. **Insets show representative SDS-PAGE gels. S, saline; LD, L-DOPA; W, WIN55212-2; A, AM251.**

* $p<0.05$, compared to vehicle-treated controls; ## $p<0.01$: compared to L-DOPA+vehicle. ANOVA followed by Tukey's post hoc.

Table 1

Enzymatic activity in dyskinetic rats after the last L-DOPA injection (in min)

Protein	saline	L-DOPA	L-DOPA+WIN
PKA (60 min)	100±3.6	128.6±12.1*	95.69±6.6
PP2A (60 min)	100.6±6.1	99.52±4.7	92.55±5.3
PP2A (120 min)	101±7.1	81.6±13.1	100.2±16.4
PP2B (60 min)	101.1±22.9	100.6±23	100.9±14.9
PP2B (120 min)	100.7±6	98.76±7.8	96.98±38

Data are expressed as mean±sem (% of control values)

Table 2

DARPP-32 phosphorylation in dyskinetic rats 60 min after L-DOPA injection

Protein	saline	L-DOPA	L-DOPA+WIN
pThr34	100±4.2	120.4±13.2	100±43.8
pThr75	100±7.1	120.4±13.1	104.5±4.8

Data are expressed as mean±sem (% of control values)

Table 3

DARPP-32 phosphorylation in dyskinetic rats 120 min after L-DOPA injection

Protein	saline	L-DOPA	L-DOPA+WIN	L-DOPA+WIN + AM251
pThr34	100±6.7	148.6±18.6 [*]	79.8±8.5	99.3±19.3 ^{##}
pThr75	100.8±17.2	91.1±6.3	85.8±6.4	96.4±13.3

Data are expressed as mean±sem (% of control values).

^{*} P<0.05 (compared to saline).^{##} P<0.01 (compared to L-DOPA).