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Anti-dyskinetic effects of cannabinoids in a rat model of Parkinson's disease: Role of CB_1 and TRPV1 receptors

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Abstract

Levodopa is the most commonly prescribed drug for Parkinson's disease (PD). Although levodopa improves PD symptoms in the initial stages of the disease, its long-term use is limited by the development of side effects, including abnormal involuntary movements (dyskinesias) and psychiatric complications. The endocannabinoid system is emerging as an important modulator of basal ganglia functions and its pharmacologic manipulation represents a promising therapy to alleviate levodopa-induced dyskinesias. Rats with 6-OHDA lesions that are chronically treated with levodopa develop increasingly severe axial, limb, locomotor and oro-facial abnormal involuntary movements (AIMs). Administration of the cannabinoid agonist WIN 55,212-2 attenuated levodopa-induced axial, limb and oral AIMs dose-dependently via a CB_1-mediated mechanism, whereas it had no effect on locomotive AIMs. By contrast, systemic administration of URB597, a potent FAAH inhibitor, did not affect AIMs scoring despite its ability to increase anandamide concentration throughout the basal ganglia. Unlike WIN, anandamide can also bind and activate transient receptor potential vanilloid type-1 (TRPV1) receptors, which have been implicated in the modulation of dopamine transmission in the basal ganglia. Interestingly, URB597 significantly decreased all AIMs subtypes only if co-administered with the TRPV1 antagonist capsazepine. Our data indicate that pharmacological blockade of TRPV1 receptors unmasks the anti-dyskinetic effects of FAAH inhibitors and that CB_1 and TRPV1 receptors play opposite roles in levodopa-induced dyskinesias.

Keywords: Anandamide; Endocannabinoid; Vanilloid; Levodopa; Dyskinesias; 6-OHDA; Basal ganglia

Introduction

Since its introduction, L-3,4-dihydroxyphenilalanine (levodopa) has remained the mainstay treatment for PD. Although levodopa alleviates parkinsonian symptoms, its long-term administration is accompanied by fluctuations in its duration of action and disabling motor complications (dyskinesias) (Obeso et al., 2004). Levodopa-induced dyskinesias (LID) are characterized by choreiform and dystonic movements and are classified according to their temporal profile as "peak-dose" (occurring at peak levodopa concentration in the brain), "diphasic" (at the beginning and end of dosing) and "off" dyskinesias (when levodopa concentration is low) (Fahn, 2000; Nutt et al., 1992).

Multiple factors have been shown to contribute to development of LID, including pulsatile stimulation of postsynaptic dopamine receptors (Westin et al., 2006), maladaptive changes in synaptic plasticity (Cenci and Lundblad, 2006; Picconi et al., 2003), neurochemical disturbances and fluctuations of levodopa/dopamine levels (Carta et al., 2006; de la Fuente-Fernandez et al., 2004; Meissner et al., 2006) and altered trafficking of NMDA receptor subunits (Fiorentini et al., 2006; Gardoni et al., 2006).

In rodents, LID can be modeled via intracerebral injection of the neurotoxin 6-OHDA – which damages the nigro-striatal pathway – followed by chronic administration of low doses of levodopa, which causes characteristic AIMs and dyskinesias-associated cellular responses (Lundblad et al., 2004). This

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model has been pharmacologically validated and represents a cost-efficient alternative to non-human primates for screening drugs with potential anti-dyskinetic properties (Lundblad et al., 2002).

Experimental evidence points to the endocannabinoid system as a novel pharmacological target to treat levodopa-associated motor disturbances (Ferrer et al., 2003; Sieradzan et al., 2001; van der Stelt et al., 2005). This system consists of a family of signaling lipids (endocannabinoids) and their allied cannabinoid receptors (Mackie, 2005; Piomelli et al., 2000). In particular, CB_1 cannabinoid receptors are highly expressed in brain areas regulating motor functions, including the basal ganglia, cerebellum and sensori-motor cortex (Mackie, 2005). In rodents, activation of dopamine receptors is accompanied by release of the endocannabinoid anandamide (AEA) throughout the basal ganglia (Ferrer et al., 2003; Giuffrida et al., 1999). Dopamine-dependent AEA elevation may serve as an inhibitory feedback to counter dopamine-mediated motor behaviors (Giuffrida et al., 1999; Beltramo et al., 2000) and is disrupted after damaging the nigro-striatal pathway with 6-OHDA (Ferrer et al., 2003), suggesting that alterations in endocannabinoid transmission may affect the dopamine–endocannabinoid crosstalk and eventually result in motor disturbances. In keeping with this hypothesis, administration of the cannabinoid agonist WIN 55,212-2 (WIN) to rats with 6-OHDA lesions ameliorates levodopa-induced oral AIMs via a CB_1-dependent mechanism (Ferrer et al., 2003).

In addition to CB_1 receptors, AEA can bind, although with low affinity, to the ionotropic transient receptor potential vanilloid subtype 1 (TRPV1) (Caterina et al., 1997; Ross, 2003). These receptors are co-expressed with CB_1 receptors in the striatum and globus pallidus (GP) (Cristino et al., 2006; Toth et al., 2005), and functional interactions between these two receptor types have been reported *in vitro* (Hermann et al., 2003) and *in vivo* (Kim et al., 2005).

In this study, we investigated the effects of the cannabinoid agonist WIN on levodopa-induced AIMs in rats with 6-OHDA lesions and tested whether AEA elevation – via pharmacological blockade of its catabolism – produced anti-dyskinetic effects similar to those observed with WIN via CB_1- and/or TRPV1-mediated mechanisms.

Materials and methods

Chemicals

Fatty acyl chlorides (5,8,11,14-eicosatetraenoylchloride, hexadecanoylchloride and 9-*cis*-octadecenoylchloride) were from Nu-Check Prep (Elysian, MN). [$^2H_4$]-Labeled ethanolamine (98% isotopic atom enrichment) from Cambridge Isotope Laboratories (Andover, MA). [$^2H_5$]-Labeled 2-AG (98% isotopic atom enrichment), AM251 and URB597 from Cayman Chemical (Ann Arbor, MI). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) from Supelco (Bellefonte, PA). Halothane from Halocarbon (River Edge, NJ). All organic solvents from Honeywell/Burdick and Jackson (Muskegon, MI). Desipramine hydrochloride, levodopa methyl ester, 6-hydroxydopamine (6-OHDA) hydrochloride and *S*-(−)carbidopa from Sigma Chemicals Co. (St. Louis, MO); WIN 55,212-2 mesylate and capsazepine from Tocris Bioscience (Ellisville, MI).

Animals and 6-OHDA lesion

Animal care and experiments were conducted in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Male Wistar rats (225–250 g; Charles River Laboratories, Wilmington, MA) were housed on a 12-h dark–light cycle, at 22±1 °C with food and water available *ad libitum*. Animals were habituated to the housing conditions for 1 week before the experiments.

DA-denervating lesions were performed by unilateral injection of 6-OHDA into the left medial forebrain bundle (MFB) as previously reported (Lundblad et al., 2002). Briefly, after intraperitoneal (i.p.) administration of desipramine (25 mg/kg, 30 min before surgery), 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 solution and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA). 6-OHDA (4 μg/μl) was dissolved in 0.2% ascorbate saline. The 6-OHDA solution (2 μl) or a corresponding volume of saline (sham lesion) were injected into the left MFB at a flow rate of 0.5 μl/min using a 10 μl Hamilton microsyringe with a 30-gauge needle at the following coordinates: AP −4.3, ML +1.6, DV −8.3 tooth bar −2.4 (relative to bregma and midline, in mm) (Paxinos and Watson, 1998). Two weeks after the lesion, the rats were screened for apomorphine-induced (0.5 mg/kg, s.c.) contralateral rotation to assess the efficacy of the lesion. Net contralateral turns were calculated by subtracting the number of ipsilateral from contralateral rotations. Only rats displaying more than 300 rotations per 30 min (corresponding to about 90% depletion of tyrosine hydroxylase (TH) positive neurons in the SNc (Ferrer et al., 2003)) were included in the study. Two weeks after the apomorphine challenge, animals were treated with a daily injection of levodopa (6 mg/kg, i.p.) plus carbidopa (12 mg/kg, i.p.) for up to 12 days. Chronic administration of this dose of levodopa has been shown to induce a gradual development of dyskinetic-like movements in the majority of rats with 6-OHDA lesions (Lundblad et al., 2002). About 37% of these animals did not develop dyskinesias and were not included in the study.

Behavioral assays

Catalepsy

WIN-induced catalepsy was determined using the bar test. Catalepsy was assessed by placing both forepaws of the animal on a horizontal bar, 13 cm above the surface, and by measuring the latency to initiate movement using a cut-off time of 60 s. On the day of the experiment, rats were administered vehicle (saline/PEG/Tween-80, 90:5:5 v/v/v, i.p.) or increasing doses of WIN (0.5, 1, 2.5 mg/kg, i.p.) 15 min before testing. Animals were