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Fatty acid amide hydrolase inhibition for the symptomatic relief of Parkinson's disease

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ABSTRACT

Elements of the endocannabinoid system are strongly expressed in the basal ganglia where they suffer profound rearrangements after dopamine depletion. Modulation of the levels of the endocannabinoid 2-arachidonoyl-glycerol by inhibiting monoacylglycerol lipase alters glial phenotypes and provides neuroprotection in a mouse model of Parkinson's disease. In this study, we assessed whether inhibiting fatty acid amide hydrolase could also provide beneficial effects on the time course of this disease. The fatty acid amide hydrolase inhibitor, URB597, was administered chronically to mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid (MPTPp) over 5 weeks. URB597 (1 mg/kg) prevented MPTPp induced motor impairment but it did not preserve the dopamine levels in the nigrostriatal pathway or regulate glial cell activation. The symptomatic relief of URB597 was confirmed in haloperidol-induced catalepsy assays, where its anti-cataleptic effects were both blocked by antagonists of the two cannabinoid receptors (CB_1 and CB_2), and abolished in animals deficient in these receptors. Other fatty acid amide hydrolase inhibitors, JNJ1661010 and TCF2, also had anti-cataleptic properties. Together, these results demonstrate an effect of fatty acid amide hydrolase inhibition on the motor symptoms of Parkinson's disease in two distinct experimental models that is mediated by cannabinoid receptors.

1. Introduction

Different elements of the endocannabinoid system (ECS) are expressed strongly in the basal ganglia, where they are involved in motor control (Fernandez-Ruiz, 2009; Fride and Mechoulam, 1993; Herkenham et al., 1991). Profound rearrangements of the ECS have been described after dopamine (DA) depletion (Pisani et al., 2011). Indeed, in experimental models of Parkinson's disease (PD) there is more mRNA encoding the cannabinoid receptor type 1 (CB_1), the CB_1 receptor-mediated signaling is enhanced (Lastres-Becker et al., 2001; Romero et al., 2000), the levels of anandamide (N-arachidonoyl-ethanolamine, AEA) increase in the external segment of the globus pallidus (GPe) and in the striatum (Di Marzo et al., 2000; Gubellini et al., 2002; van der Stelt et al., 2005), and fatty acid amide hydrolase (FAAH) activity is reduced (Gubellini

Abbreviations: 2-AG, 2-arachidonoyl-glycerol; AEA, anandamide; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CNS, central nervous system; DOPAC, 3,4-Dihydroxyphenylacetic acid; DA, dopamine; D_2, dopamine receptor subtype 2; ECS, endocannabinoid system; eCBs, endocannabinoids; FAAH, fatty acid amide hydrolase; GFAP, glial fibrillary acidic protein; GPe, external segment of the globus pallidus; HVA, homovanillic acid; Iba-1, ionized calcium-binding adapter molecule 1; LTD, long-term depression; MAGL, monoacylglycerol lipase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPTPp, MPTP plus probenecid; NAEs, N-acylethanolamines; OEA, oleoylethanolamine; PEA, palmitoylethanolamine; PD, Parkinson's disease; SNpc, *substantia nigra pars compacta*; TH, tyrosine hydroxylase; TRPV1, transient receptor potential vanilloid-type 1.

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et al., 2002). Significantly, DA-replacement therapy returns the elevated AEA levels to control levels in PD patients (Pisani et al., 2010). Regardless of whether these changes contribute to movement suppression or are compensatory mechanisms to counterbalance DA depletion, it is clear that the ECS is affected in parkinsonian conditions and its modulation is a promising means to improve PD motor symptoms and/or PD-related neurodegeneration.

Administration of cannabinoids induces a wide range of effects in both, PD animal models and patients (Kluger et al., 2015). Although CB_1 receptor agonists potentiate bradykinesia in the MPTP primate model of PD (Meschler et al., 2001), studies in the 6-OHDA rat model and in the marmoset model have shown that such agonists improve motor impairment (Sanudo-Pena et al., 1998; van Vliet et al., 2008). On the other hand, CB_1 receptor antagonists have shown to consistently improve amphetamine-induced turning behavior, hypokinesia and bradykinesia in the 6-OHDA rat model (Cao et al., 2007; Fernandez-Espejo et al., 2005; Gonzalez et al., 2006; Kelsey et al., 2009) and to enhance the antiparkinsonian action of levodopa (L-DOPA) in the MPTP monkey model (Cao et al., 2007; Fernandez-Espejo et al., 2005; Gonzalez et al., 2006; Kelsey et al., 2009). In humans, there is evidence that cannabinoids may improve motor and non-motor symptoms of (Chagas et al., 2014a; Lotan et al., 2014; Zuardi et al., 2009), although benefits for motor symptoms are not always seen and there have been mixed results regarding L-DOPA-induced dyskinesia (Carroll et al., 2004; Chagas et al., 2014b; Mesnage et al., 2004; Sieradzan et al., 2001). Both agonists and antagonists of CB_1 receptors modulate the effects of L-DOPA, the main drug used in replacement therapy, and they may therefore be useful to treat dyskinesia (Martinez et al., 2012; Segovia et al., 2003).

Cannabinoids are considered to be neuroprotective agents, and they appear to exert their neuroprotective effects in animal models of PD through cannabinoid receptor-dependent and -independent mechanisms (Garcia et al., 2011; Lastres-Becker et al., 2005; Price et al., 2009). Hence, to identify ECS-modulating agents capable of providing therapeutic or neuroprotective benefits in patients with movement disorders, it is necessary to better understand cannabinoids mode of action, pharmacology and pharmacokinetics.

AEA and 2-arachidonoyl-glycerol (2-AG) are endogenous ligands of cannabinoid CB_1 and CB_2 receptors (Devane et al., 1992; Di Marzo et al., 2002; Mechoulam et al., 1995). The level of these endocannabinoids (eCB) in the central nervous system (CNS) is influenced by two catabolic enzymes, FAAH and monoacylglycerol lipase (MAGL) (Cravatt et al., 1996; Nomura et al., 2008). Selective and efficacious inhibitors of FAAH and MAGL have been developed, and they produce a specific increase in eCB concentrations *in vivo* (Fegley et al., 2005; Long et al., 2009). Thus, inhibiting these enzymes constitutes an indirect strategy to modulate the ECS and this approach may have better outcomes than the use of cannabinoid receptor ligands (Chhatwal et al., 2005; Gaetani et al., 2003; Marsicano et al., 2003; Van Sickle et al., 2005). Specific inhibition of MAGL with JZL184 has a neuroprotective effect in animal models of PD due to the activation of a neuroprotective glial phenotype and a decrease in prostaglandin synthesis (Fernandez-Suarez et al., 2014; Nomura et al., 2011). Inhibition of FAAH has been investigated to characterize the modulation of DA receptor-mediated motor responses by eCBs (Kreitzer and Malenka, 2007). However, the specific effect of FAAH inhibition on the motor symptoms of PD remains to be defined. Thus, this study was set out to determine whether enhancing AEA levels by inhibiting FAAH has beneficial effects on PD symptoms and/or prevents PD-related neurodegeneration. In the chronic MPTP mouse model, a five-week treatment with a specific FAAH inhibitor, URB597, significantly improved the motor symptoms of these animals without any sign of nigral neurorestoration. The symptomatic effect was further characterized in haloperidol-induced cataleptic animals treated with cannabinoid and vanilloid receptor antagonists, and it was shown that CB_1 and CB_2 but not the transient receptor potential vanilloid-type 1 (TRPV1) receptors were involved in the reversal of catalepsy provoked by different FAAH inhibitors. These results point to FAAH inhibition as a promising non-dopaminergic therapeutic strategy for the symptomatic treatment of PD.

2. Materials and methods

2.1. Animals

Adult (3 month old) male C57BL/6J mice (25–30 g) age were obtained from Charles River (Barcelona, Spain) and 5 mice per cage were housed at 21 °C in a humidity-controlled environment, on a 12/12 h light/dark cycle (lights on at 8 am), with *ad libitum* access to food and water. All the procedures involving animals were carried out in accordance with the EU Directive 2010/63/EU governing the care and use of laboratory animals. The experimental design was approved by the Ethical Committee for Animal Testing of the University of Navarra. Male, 7 month old CB_1 receptor knockout (KO) mice ($CB_1^{-/-}$) and their wild type ($CB_1^{+/+}$) littermates (24–26 g), and male CB_2 receptor knock-out mice ($CB_2^{-/-}$) and their wild type ($CB_2^{+/+}$) littermates (30–35 g) of the same age were also used in these experiments. The mice lacking CB_1 cannabinoid receptor were generated on a C57BL/6N congenic background, as described previously (Marsicano et al., 2002). The CB_1 KO mouse on a C57BL/6N genetic background was kindly donated by Giovanni Marsicano (Marsicano et al., 2002). These animals were backcrossed to C57BL/6NCrl for 8 generations and generated by heterozygous breeding to avoid possible genetic differences in maternal care. The CB_2 KO mice on a C57BL/6J genetic background were kindly provided by Nancy E. Buckley (Cal. State Polytechnic University, Pomona, CA) (Buckley et al., 2000). These animals were backcrossed to C57BL/6J for 3 generations and share 93.75% of genetic background with their congenic strain.

2.2. Chronic MPTP mouse model

To induce a bilateral and progressive but partial dopaminergic lesion, mice received 10 intraperitoneal (i.p.) injections of MPTP hydrochloride (20 mg/kg in saline: Sigma-Aldrich, St. Louis, MO, USA) plus probenecid (250 mg/kg in saline: Invitrogen, Paisley, UK), administered twice a week over 5 weeks (Fig. 1A). MPTP and probenecid (MPTPp) were administered in two consecutive injections, while control animals received probenecid and saline (Braun, Barcelona, Spain) on an identical administration regime. To be able to compare the effect of FAAH inhibition with MAGL inhibition in MPTPp treated mice the administration regime of the inhibitor was the same used previously (Fernandez-Suarez et al., 2014). This regime (Fig. 1A) was selected after observing in a pilot experiment that animals receiving MPTPp treatment for 5 weeks and daily injections of the inhibitor got ulcers in the area of administration. Therefore, one-half of the MPTPp-treated animals and one-half of the control animals were i.p. injected with 1 mg/kg URB597 (Merck Millipore, Darmstadt, Germany) 5 days per week along the 5 weeks of MPTPp treatment (Fig. 1A). The second half of animals was treated with the vehicle of URB597, i.e. with saline containing 15% dimethylsulfoxide (DMSO, Sigma-Aldrich), 5% PEG (Sigma-Aldrich) and 5% Tween-80 (Guinama, Valencia, Spain). Mice receiving 5 doses of the inhibitor per week did not show any obvious alteration in the administration areas. Mice were sacrificed 48 h after the last MPTPp administration (40 h after the last URB597/vehicle administration).

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