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Review Article

The Use of Cannabinoids in Parkinson's Disease

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Abstract. Parkinson's disease (PD) is a very common neurodegenerative disorder in the elderly for which there is no current cure. The neuropathological hallmark is the loss of dopaminergic cells in the substantia nigra pars compacta. Current treatments use L-DOPA and dopamine agonists to replace the lack of dopamine, however such treatments have significant limitations and side effects, thus, the need for more effective therapeutics is critical. Cannabinoids (CBs), which include Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin, target the endocannabinoid (ECB) system, which is highly involved in dopaminergic functions. The endocannabinoid system undergoes extensive changes in PD such as upregulation of the ECB anandamide, in addition to variations in the concentration of CB receptors. These changes can be modified and corrected using CB1 and CB2 receptor ligands and by modulating the levels of the ECB catabolic enzyme fatty acid amide hydrolase (FAAH), in order to increase endogenous anandamide (AEA) levels. Therefore, CBs may represent a valid therapeutic alternative to treat PD. CB drugs may not only treat the symptoms of the disease, but may also help slow down disease progression. Nevertheless, with regards to motor symptoms of PD such as rigidity, bradykinesia, postural instability, resting tremors and levodopa-induced dyskinesia, evidence of the therapeutic effect of CBs is somewhat inconsistent. Although only evidence in the preclinical phase, more promising results have been seen in general regarding the neuroprotective effect of CBs, as well as in relation to sleep, depression and pain.

Keywords: Parkinson's disease, exocannabinoids, the endocannabinoid system, dyskinesia, dopamine

1 Introduction

Cannabis is derived from a plant named Cannabis sativa and has been used as a therapeutic agent since ancient times; it was clinically recognized as a medicinal compound in the mid-19th century (Borgelt, Franson, Nussbaum & Wang, 2013), following research done by Sir William B. O'Shaugnessey (Gowran, Noonan & Campbell, 2010). Medical cannabis has been widely used in medicine to treat an extensive range of clinical problems such as inflammation, oxidative stress, spasticity and rheumatism, as well as pain (Gowran et al., 2010; Babayeva, Assefa, Basu, Chumki & Loewy, 2016; Fitzcharles & Eisenberg, 2018) and Parkinson's disease (PD).

Affecting 1% of the population, PD is the second most common neurodegenerative disorder, preceded by Alzheimer's disease (Xu, Kochanek, Murphy & Tejada-Vera, 2010). It is estimated that by the year 2030 there will be approximately nine million cases of PD across the fifteen highest populated countries in the world (Dorsey et al., 2006). Today Parkinsonian patients are only treated with substitutive therapy to compensate the lack of dopamine in their brain. Therefore, such treatment is purely symptomatic and does not affect the progression of the disease, thus there is currently no cure for PD (Babayeva et al., 2016). Moreover, adverse effects appear several years after initiation of treatment (as shown by the graph in Fig. 1) and can include motor fluctuations and dyskinesia (L-DOPA induced dyskinesia: LID) (Marsden & Parkes, 1977). This review aims to highlight the effects of such CBs on patients suffering from PD.

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1.1 The Phyto-, Exo- and the Endocannabinoids

The products extracted from the cannabis plant consist of a large number of phytocannabinoids (phyto-CBs), a term used to differentiate cannabinoids derived from the plant, from those found in the body (i.e., endocannabinoids, ECBs) or those chemically synthesized (i.e., exocannabinoids, exo-CBs). Of about 85 phyto-CBs contained in the plant there are two significant ones, namely cannabidiol (CBD) and delta-9-Tetrahydrocannabinol (Δ9-THC) (Russo, 2011; Babayeva et al., 2016). Synthetic cannabinoids were made for cannabinoid research (exo-CBs). Exo-CBs encompass a variety of distinct chemical classes: the classical cannabinoids structurally related to THC, the non-classical cannabinoids (cannabimimetics) including the aminoalkylindoles, 1,5-diarylpyrazoles, quinolines, and arylsulfonamides, as well as eicosanoids related to endocannabinoids (Lambert & Fowler, 2005). Synthetic cannabinoids were needed partly due to legal restrictions on natural cannabinoids, which make them difficult to obtain for research. At the time of the discovery of the cannabinoid CB1R, there were just two main chemical classes of psychotropic cannabinoids: the 'classical cannabinoids' that consist of tricyclic dibenzopyrans, such as Δ9-THC and its far more potent synthetic analogue (−)-11-hydroxy-Δ8-THC-dimethylheptyl (HU-210), as well as 'nonclassical' cannabinoids of which the bicyclic CP55940 is an important member. Subsequently, other chemical classes of psychotropic cannabinoids made their appearance, including the aminoalkylindole R-(+)-WIN55212, endogenous eicosanoids such as anandamide and 2-arachidonoyl glycerol (see below), in addition to the more recently discovered, Bayer compound, BAY 38-7271. Some early synthetic cannabinoids were also used clinically. Nabilone, a first-generation synthetic THC analog, has been used as an antiemetic to combat vomiting and nausea since 1981. Synthetic THC (Marinol, dronabinol) has been used as an antiemetic since 1985 and an appetite stimulant since 1991 (Pertwee, 2006). The CB1R inverse agonist SR141716 (also known as rimonabant), was discovered and developed by Sanofi-Aventis and temporally approved as an anorectic anti-obesity drug, but was later withdrawn in 2008 due to serious psychiatric side effects including suicide (Sam, Salem & Ghatei, 2011).

In the early 2000s, synthetic cannabinoids began to be used for recreational drug use in an attempt to achieve similar effects to that of cannabis. The likelihood of severe abuse and addiction produced by SCs are of concern for the scientific community who are also interested in the potential therapeutic value of cannabinoids (Le Boisselier, Alexandre, Lelong-Boulouard & Debruyne, 2017).

The ECBs consists of several components: lipid-based molecules called ECBs, CB receptors, transport proteins, as well as enzymes responsible for the synthesis and degradation of the ECBs (Rodríguez de Fonseca et al., 2005). Synthesis enzymes include N- arachidonoyl-phosphatidylethanolamine (NAPE)-specific phospholipase D and diacylglycerol lipase-a (DAGLa), while those that catabolize the ECBs include fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) (Stampanoni Bassi, Sancesario, Morace, Centonze & Iezzi, 2017). The body has several ECBs, the most significant being anandamide (N- arachidonoylethanolamine or AEA) and 2-arachidonoyl-glycerol (2-AG). FAAH is responsible for the degradation of AEA, while MAGL degrades 2-AG (Gowran et al., 2010). The ECB system functions to modulate locomotion, memory, feeding behaviour, analgesia, energy balance and metabolism, stress response, sleep and many other processes (Pacher, 2006).

AEA and 2-AG mainly bind to two types of G-protein coupled receptors (GPCRs): CB1 and CB2 receptors (Gowran et al., 2010). They also bind to a lesser extent to other receptors, such as GPCR 55 (GPR55) (Ross, 2009) and GPCR 18 (GPR18) (McHugh et al., 2010). These are orphan receptors that are involved in the assembly and mobilization of microglia, and the regulation of neuropathic pain (Gowran et al., 2010). CBs can also bind to transient receptor potential cation channel subfamily V member 1 (TRPV1) (Tóth, Blumberg & Boczán, 2009), which plays a role in inflammation and pain (Costa et al., 2010), in addition to the abnormal cannabidiol receptor (McHugh et al., 2010) and the peroxisome proliferator-activated receptor (PPAR) (O'Sullivan, 2009). The function of GPR55, GPR18 and TRVP1 highlights them as potential targets in diseases involving neuropathic pain and neuroinflammation (Gowran et al., 2010). CB receptors are extensively found in the body, but are mainly concentrated in the central nervous system (CNS) and the immune system. CB1 receptors are differentially distributed in the CNS (Herkenham, Lynn, Johnson & Melvin, 1991), whilst CB2 receptors are mostly found in the immune system (Gowran et al., 2010), on cells like T-cells, B-cells and monocytes, and in organs such as the spleen. In the CNS, CB2 receptors are restricted to the brainstem (Van Sickle, 2005) and on microglia (Núñez et al., 2008). CB receptors can also be found to a lesser extent in the peripheral nervous system, reproductive organs, cardiovascular system and the gastrointestinal system (Babayeva et al., 2016).

The ECB 2-AG is 150 times more abundant in the brain than AEA and it binds to CB receptors much more potently (Buczynski & Parsons, 2010). It is the main ECB released in the midbrain in a calcium ion

dependent fashion. ECBs regulate synaptic transmission via "retrograde signaling"; one important feature of these ECBs is that they are synthesized *on demand*. Once ECBs are synthesized on the post-synaptic membrane, they travel across the synaptic cleft to bind to CB1 receptors on the presynaptic elements, where they cause cessation of neurotransmitter release (Gowran et al., 2010). This is possible because, on binding to the receptor (which is associated with a specific Golf protein), ECBs inhibit voltage-gated calcium channels and increase the activity of inward rectifying potassium channels in the presynaptic cell (Lévénès, Daniel, Soubrié & Crépel, 1998; Kreitzer & Regehr, 2001; Reggio, 2010). The calcium channel can be of the L-type, Q/P-type and N-type (Melis & Pistis, 2007). In this way, ECBs may protect against excessive excitation or inhibition (Lovinger, 2008). ECBs prevent the release of GABA on binding to CB1 receptors; this process is thus known as depolarization induced suppression of inhibition (DSI). The same process occurs in glutaminergic neurons, but in this case the process is called depolarization induced suppression of excitation (DSE) (Alger, 2002; Heinbockel, 2005). Serotonin (5-HT), acetylcholine and opioid peptides are other examples of neurotransmitters that can be modulated via CBs (Heifets & Castillo, 2009). Following activation of its receptor, the ECB is removed from the synapse via AEA membrane transporters (Melis & Pistis, 2007).

2 Effect of Some Cannabinoids on the Endocannabinoid System

2.1 Δ9-tetrahydrocannabinol

Δ9-THC is a partial agonist of both CB1 and CB2 receptors. The strength of the response it generates on binding to receptors depends on the concentration of receptors and the efficiency of their signalling. It is also affected by continuous liberation of ECBs (Pertwee, 2008). The chemical structure of Δ9-THC resembles that of AEA, implying that it mimics the effect of ECBs. It has been shown to enhance appetite and cause smooth muscle relaxation (Petrocellis, Cascio & Marzo, 2004). Smoking Δ9-THC results in the immediate onset of its effects, since the drug enters the circulation directly from the lungs. These effects become apparent within minutes and are known as a "high". Since Δ9-THC exerts its effect on cannabinoid receptors, which are highly concentrated in the CNS, the smoker experiences changes in conscious perception, euphoria and feelings of well-being. This occurs because the ECB system is over-stimulated (Basavarajappa, 2007). When used short term, Δ9-THC also influences the dopaminergic system by stimulating dopamine release, however, it has the opposite effect if used chronically (Bloomfield, Ashok, Volkow & Howes, 2016).

2.2 Cannabidiol

Cannabidiol (CBD) has different effects on the CB system as it binds to CB1 and CB2 receptors sparingly, but it is still able to block the action of Δ9-THC indirectly. This would theoretically diminish the effect of Δ9-THC. However, the effect is the opposite, as CBD causes upregulation of CB1 receptors (Devinsky et al., 2014). Its influence on the action of Δ9-THC gives it the ability to control several side effects associated with Δ9-THC, such as tachyarrhythmia and increased appetite (Russo, 2011). CBD also supplements some of Δ9-THC's desired outcomes by helping to reduce its psychotic effects, making it more tolerable to the patient and increasing its therapeutic window (Karniol & Carlini, 1973). CBD has also been shown to help with psychosis by preventing ECBs from being catabolized, thus prolonging their activity (Leweke et al., 2012). CBD can also act as an inverse agonist of the CB2 receptor, which allows it to prevent immune system cells from migrating (Pertwee, 2008). CBD delays the uptake of the neurotransmitter adenosine, thus increasing the levels of adenosine A1 receptor activation in the brain, which neutralizes a portion of the effects of activation of CB1 receptors (Devinsky et al., 2014).

2.3 Δ9-tetrahydrocannabivarin

Δ9-tetrahydrocannabivarin (Δ9-THCV) is another cannabinoid extracted from marijuana which mimics Δ9-THC. Like Δ9-THC, it binds with equal efficacy to both CB1 and CB2 receptors, but its effects are several times less potent. *In vitro*, it is a CB2 receptor partial agonist, while *in vivo* it is a CB1 receptor agonist (it is a CB1 receptor antagonist at lower concentrations). However, in tissues that contain CB1 receptors, it blocks cannabinoid receptor agonists (Pertwee, 2008).

3 Parkinson's Disease

Neurodegenerative disorders, which include PD, have also been extensively studied in the context of finding cannabinoid (CB)–based therapy (Gowran et al., 2010). The neurodegeneration associated with this disease occurs in dopaminergic cells of the nigrostriatal system. The main functional component of the basal ganglia affected is the substantia nigra pars compacta (SNc), located in the midbrain. As a result, there is a lack of dopamine in the striatum, leading to less excitation of the direct striatonigral pathway that facilitates movement, and less inhibition of the indirect pathway that antagonizes movement. Degeneration of the SNc neurons occurs over a prolonged period and can take up to several decades (Galvan & Wichmann, 2008). The cause of this is not well known but has been linked to both environmental factors, such as pesticides and certain antipsychotics, as well as genetic factors, such as mutations

in certain genes that encode proteins, like PINK1 and parkin (Gowran et al., 2010).

PD has both motor and non-motor symptoms. The non-motor symptoms appear before the motor symptoms, with the clinical diagnosis made after there is a significant loss of nigrostriatal dopamine (about 70%) (Bernheimer, Birkmayer, Hornykiewicz, Jellinger & Seitelberger, 1973). Since dopamine facilitates movement, PD patients exhibit hypokinesia, along with other motor symptoms such as bradykinesia and akinesia, muscle stiffness, and resting tremor (Thomas & Beal, 2007; Galvan & Wichmann, 2008; Rodriguez-Oroz et al., 2009). Non-motor symptoms that may be experienced include low blood pressure on pertaining an upright posture, depression, difficulty in defecation, anxiety and sleep disorders. When the disease has progressed significantly, dementia may also occur (Velseboer, de Haan, Wieling, Goldstein & de Bie, 2011; Connolly & Fox, 2013; Stocchi et al., 2014). At present, the common approach towards the treatment of motor symptoms involves dopamine replacement therapy (Goetz & Pal, 2014). This is achieved via several means, the main one being administration of dopamine precursor levodopa (L-DOPA) (Pantcheva, Reyes, Hoover, Kaelber & Borlongan, 2015). Other approaches are: (1) limiting dopamine break down with catechol-O- methyltransferase (COMT) inhibitors, (2) stimulating dopamine receptors with dopamine agonists (i.e., Sinemet), and (3) enhancing dopamine release while preventing reuptake of dopamine at presynaptic terminals (Lees, 2005; Goetz & Pal, 2014). Although effective, the efficacy of L-DOPA treatment tends to decrease when used chronically and may also cause significant side effects. Furthermore, L-DOPA only caters specifically to the motor symptoms of the disease as non-motor symptoms do not respond to this type of treatment (Pantcheva et al., 2015).

Certain drugs, such as monoamine oxidase B (MAO-B) inhibitors and COMT inhibitors, are effective in limiting the adverse effects caused by L-DOPA. In addition to these, dopamine agonists, apart from being used in early stages to treat the disease, are also useful in decreasing the motor fluctuations associated with L-DOPA therapy (Pahwa et al., 2006). The various difficulties associated with the forms of therapy mentioned has initiated research into alternative approaches, among them, medical marijuana. Several studies have shown that different phyto-CBs can be used to treat various symptoms that accompany PD, including both motor and non-motor symptoms (Babayeva et al., 2016), by counteracting oxidative stress, neuroinflammation and excitotoxicity (Fernández-Ruiz et al., 2013).

3.1 The Endocannabinoid System and Dopamine

The ECB system is central in regulating dopaminergic transmission and motor functions, hence it is highly expressed in the basal ganglia (El Manira & Kyriakatos, 2010). The globus pallidus and the SNc are components of the basal ganglia associated with locomotion and cognition, and they express large amounts of CB1 receptors and ECBs (especially AEA) (Babayeva et al., 2016). These CB1 receptor expressing neurons (including GABAergic and glutamatergic cells) are found close to, or in contact with, dopaminergic neurons. Nigrostriatal dopaminergic neurons do not contain CB1 receptors (Fernández-Ruiz, Lastres-Becker, Cabranes, González & Ramos, 2002). However, ECBs can exert their effect on dopaminergic signalling indirectly (van der Stelt & Di Marzo, 2003). Therefore, alterations in the ECB system considerably affect the nigrostriatal dopaminergic function. For example, activation of CB1 receptors on GABAergic neurons results in decreased GABA release onto dopaminergic neurons in the SNc, thus resulting in their disinhibition (Fernández-Ruiz, 2009).

On the contrary to CB1 receptors, CB2 receptors are found on dopaminergic neurons (M. García, Cinquina, Palomo-Garo, Rábano & Fernández-Ruiz, 2015). This indicates an immediate relationship between dopaminergic signalling and the ECB system. Also, some ECBs may be produced by the soma and dendrites of the dopaminergic cells themselves, which would act on the pre-synaptic cell as described previously, hence neurotransmitter release onto dopaminergic cells would be reduced (Riegel, 2004). The major factor promoting ECB release from dopaminergic cells is electrical stimulation of the aforementioned dopaminergic cell. This eventually causes the release of calcium ions from intracellular stores, thus promoting ECB release. Suppression of sn1-specific diacylglycerol lipase, which is responsible for the synthesis of 2-AG, leads to the cessation of synaptic modulation. This implies that dopaminergic cells synthesize 2-AG rather than AEA (Melis, 2004).

There are other connections between the dopaminergic system and ECBs, one of them being that the receptor TRPV1, which can bind to AEA, can be found on dopaminergic neurons (Mezey et al., 2000). The CB1 receptor is capable of forming heteromeric dimers with the dopamine receptors D1 and D2, which are both G protein-coupled. Additionally, CB1 receptors and D2 receptors make use of a common pool of G-proteins, which points to overlap in intracellular signalling (Ferré, Goldberg, Lluis & Franco, 2009). It has also been shown that D2 receptors in the striatum control the production of AEA. These associations prove that there is a link between the motor activity induced by dopamine and the ECB system, with the ECB system controlling

it via a negative feedback response (Giuffrida et al., 1999). The link between the ECB system and dopaminergic, GABAergic and glutaminergic signalling makes it an appropriate focal point in the search for drugs to treat PD.

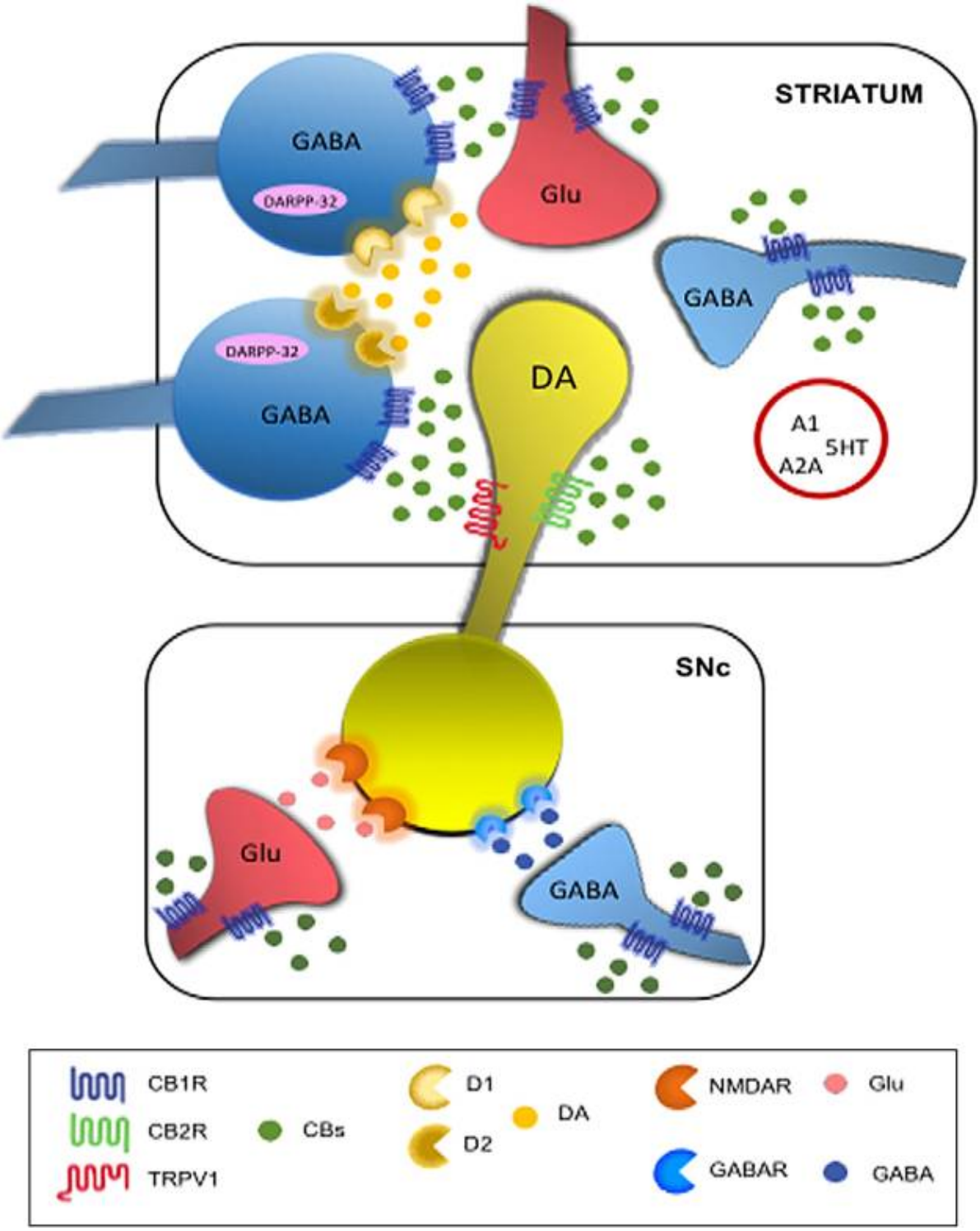

Figure 1: A schematic drawing depicting the way cannabinoids interact with the dopaminergic system in the basal ganglia. Taken from Stampanoni Bassi, Sancesario, Morace, Centonze and Iezzi (2017).

4 Alterations in the Endocannabinoid System in Parkinson's Disease

Various studies have shown that the dopamine depletion occurring in PD leads to changes in the ECB system and a disequilibrium between the direct and indirect pathways of the basal ganglia that regulate movement (V. Pisani et al., 2010).

4.1 Changes in Anandamide Levels

Neurotoxin 6-hydroxydopamine (6-OHDA) is known to mimic the lack of dopamine experienced in PD when infused into the SNc or the striatum of rodent brains; it also induced higher AEA levels (Giuffrida et al., 2004). Similar results were obtained in a group of PD sufferers (with varying degrees of severity), showing double AEA levels than that of the concentration found in control patients. It was also found that AEA levels did not vary with the severity of the disease (A. Pisani et al., 2005).

The rise in AEA might be a compensatory mechanism to make up for the loss of dopamine, as studies have shown that AEA reduces the over-activity of glutamatergic cortico-striatal pathways in rodents injected with 6-OHDA (Giuffrida et al., 2004).

4.2 Changes in CB1 Receptors

CB1 receptors are found on corticostriatal glutamatergic neurons and on GABAergic neurons innervating the medial globus pallidus, lateral globus pallidus and the SNc, as well as in excitatory projections from the subthalamic nucleus to the medial globus pallidus and the SNc (Benarroch, 2007). They are also located in striatum parvalbumin immune-reactive interneurons, cholinergic interneurons, and neurons that contain nitric oxide synthase (Fusco et al., 2004). In PD patients, basal ganglia nuclei exhibit high amounts of mRNA encoding CB1 receptors. This occurs in the intermediate and late stages of the disease when motor symptoms start appearing. In contrast, in the early stages of the disease, CB1 receptors are down-regulated (Gowran et al., 2010). Due to the lack of CB1 receptors, there is a lack of inhibition of glutamate release, which can cause excitotoxicity (van der Stelt et al., 2002). These changes can be applied to medication for PD, which will be discussed in the next paragraphs.

5 Different Approaches to Reversing Endocannabinoid Changes in PD

5.1 CB1 Receptor Agonists

Cannabinoid agonists promote hypokinesia, while cannabinoid antagonists endorse hyperkinesia. Hypokinetic effects are mediated via CB1 receptor activation (Bisogno et al., 1999). In the case of CB agonists, they can be in the form of synthetic cannabinoids, ECBs, or phyto-CB (de Lago, de Miguel, Lastres-Becker, Ramos & Fernández-Ruiz, 2004). Studies have shown that the synthetic cannabinoid HU-210 decreases LID and causes a decline in glutaminergic signalling on activation of CB1 receptors (Gilgun-Sherki, Melamed, Mechoulam & Offen, 2003). When Park-2 knockout (KO) mice (which depict a model of early-stage PD) were treated with Δ9-THC, they were reported to have high tyrosine hydroxylase levels, which is a critical enzyme in the synthesis of dopamine. They also experienced hypokinesia. However, it was of much less significance than that experienced by control mice (González et al., 2005). This is consistent with the fact that CB1 receptors are down-regulated in the early stages of the disease. Treatment with Δ9-THC and two other synthetic cannabinoids (WIN 55,212-2 and CP 55,940) has been shown to increase dopamine levels (Romero et al., 2002). Despite the undesired hypokinetic effects of cannabinoid agonists (except in the case of alleviating dyskinesia), it has been observed that they have beneficial properties when it comes to the neurodegeneration that occurs in PD. A drug named Sativex (which contains both Δ9-THC and CBD) may be used in treatment of PD, since the CBs in it have been found to act as antioxidants and protect nigrostriatal dopaminergic neurons from 6-OHDA-lesion (Lastres-Becker, Molina-Holgado, Ramos, Mechoulam & Fernández-Ruiz, 2005). This neuroprotective property suggests that cannabinoid agonists not only help treat symptoms, but also prevent the disease from progressing further.

5.2 CB1 Receptor Antagonists

In the late stages of the disease, CB1 receptor antagonists can be used to hinder the effects of high CB1 receptor density. This was investigated in a study where rodents were injected with 6-OHDA to mimic the hypokinesia experienced in PD. CB1 receptors were found to be up-regulated in these rats. The specific CB1 receptor antagonist rimonabant was found to be effective against motor inhibition. However, this was only effective when the drug was administered at small doses and was unrelated to dopamine, GABA and glutamate signalling in the striatum (González et al., 2006). In another study, PD-lesioned animals were administered varying concentrations of rimonabant and experienced an increase in glutamatergic function in the striatum, but this occurred only at the highest dose administered (of 1 mg/kg) (García-Arencibia, Ferraro, Tanganelli & Fernández-Ruiz, 2008). The combined use of rimonabant with L-DOPA diminishes the dyskinesia associated with PD (Segovia, Mora, Crossman & Brotchie, 2003). Rimonabant has also been shown to reverse the effects of Δ9-THC (Di Marzo et al., 2001), and prevent the increase in dopamine that is caused by Δ9-THC (Tanda, 1997). Despite the success in other

animal species, rimonabant is not effective in primate species (Meschler, Howlett & Madras, 2001). Other CB1 receptor antagonists, including THCV, are effective against alterations in glutaminergic transmission and prevention of hypokinesia in rodents, thus alleviating symptoms and slowing disease progression (C. García et al., 2011).

5.3 Modifying Endocannabinoid Levels

Since FAAH is the primary enzyme responsible for the degradation of AEA, FAAH inhibitors can be used to manipulate the levels of endogenous cannabinoids. Interestingly, the enzyme is already present at lower than normal concentrations in patients with PD. The FAAH inhibitor [3-(3-carbamoylphenyl)phenyl] N-cyclohexyl carbamate (URB597) has been proven to increase AEA levels, which in turn causes dopamine levels to rise (Solinas, Justinova, Golberg & Tanda, 2006). URB587 is responsible for reducing side effects associated with L-DOPA treatment, such as impulse control disorder and dopamine dysregulation syndrome, while still reaping the anti-Parkinsonian benefits of L-DOPA (Johnston et al., 2010).

5.4 CB2 Receptor Agonists

CB2 receptors have been shown to play a role in the neurological inflammation that occurs in PD. The SNc microglial cells of patients with PD express higher than normal level of CB2 receptors (Gómez-Gálvez, Palomo-Garo, Fernández-Ruiz & García, 2016). Neuroinflammation can be induced in rats by striatal injection of lipopolysaccharide (LPS) to mimic inflammation which occurs in PD. It was shown that CB2 receptor KO mice experienced an increased loss of SNc dopaminergic cells. This suggests that CB2 receptors have a neuroprotective effect. CB2 receptor KO mice also showed a higher rise in CD68 immunostaining (which corresponds to activated microglia and macrophages) than wild type animals. When the inflamed brain was treated with HU-308 (a CB2 agonist), the neuroinflammation induced by LPS was counteracted and there was a fall in CD68 immunostaining. A selective CB2 receptor agonist HU-308 has also been shown to counteract the increase in pro-inflammatory mediators caused by LPS (Gómez-Gálvez et al., 2016).

5.5 Cannabinoids and the Motor Symptoms of PD

As far as the motor symptoms of PD are concerned, studies have shown that cannabinoids are beneficial in preventing dyskinesia induced via dopamine replacement therapy (Sieradzan et al., 2001), as well as helping with PD symptoms such as resting tremors, rigidity, bradykinesia and postural instability (Consroe, Sandyk & Snider, 1986; Venderová, Ružička, Voříšek & Višňovský, 2004).

5.6 Effect of Cannabinoids on Parkinsonian Motor Symptoms

in a survey study, Venderová and colleagues (2004) found that 45.9% of a group of PD patients found cannabis helpful overall, with regards to the alleviation of Parkinsonian motor symptoms. The degree of improvement varied from mild to significant. Moreover, 30.6% experienced fewer resting tremors, 44.7% experienced an improvement with bradykinesia, and 37.7% experienced less muscular rigidity. Almost all of these patients administered cannabis orally, most often with meals and with a frequency of once every single day. Besides from one patient who had only ingested cannabis once, before the day of testing, all patients had been taking cannabis for several months. Only a small percentage of participants (4.7%) felt that cannabis made their condition worse. On average, it took approximately two months from first ingestion for patients to experience any benefits. PD patients on cannabis were tested for 11-nor-9-THCOOH (which is one of the main metabolites of Δ9-THC). It was found that those with a high concentration of this metabolite in their urine ($> 50\,\text{ng/ml}$) experienced improvements in bradykinesia and rigidity, while those with a low concentration of the metabolite in their urine ($< 50\,\text{ng/ml}$) experienced no improvements. Interestingly, the patient who had only taken cannabis on one occasion had very high levels of 11-nor-9-THCOOH, but did not experience any improvements. This suggests that chronic use is essential in order for cannabis to work (Venderová et al., 2004).

In another study, several PD patients were assessed before and after smoking marijuana, using the Unified Parkinson's Disease Rating Scale (UPDRS). Their scores improved significantly after smoking cannabis, both in patients with response fluctuations and in patients who lacked response fluctuations (Lotan, Treves, Roditi & Djaldetti, 2014).

Out of the four main motor symptoms of PD (bradykinesia, postural instability, resting tremor, rigidity), postural instability was the only one that did not improve with smoking marijuana. The effect of smoking marijuana lasted around 3 hours (Lotan et al., 2014).

5.7 Effect on L-DOPA Induced Dyskinesia

There are different hypotheses with regards to the cause of dyskinesia after dopamine replacement therapy. It has been suggested that it occurs because the lateral globus pallidus (GPl) is overexcited (Sieradzan et al., 2001). Interestingly, CB1 receptors are very numerous on the membrane of the pre-synaptic GABAergic neurons of the striatopallidal pathway (Sieradzan et al., 2001). Activation of these CB1 receptors reduces GABA release, thus increasing GPI GABAergic signalling and reversing dyskinesia. PD patients with

dyskinesia were assessed using the Rush Dyskinesia Disability Scale following intake of the CB1 receptor agonist nabilone. These patients' scores were greatly improved compared to those who had taken a placebo (Sieradzan et al., 2001), and this effect is dependent on CB1 receptors (More & Choi, 2015). Nabilone is also effective as an antidyskinetic in 1-methyl-4-phenyl-1,2,5,6 tetrahydropyridine (MPTP)-lesioned monkeys treated with L-DOPA. Furthemore, the pharmacological effects of L-DOPA were enhanced by 76% when given in conjunction with nabilone (Fox, Henry, Hill, Crossman & Brotchie, 2002). In one particular study, 14.1% of a group of PD sufferers experienced improvement of LID after intake of cannabis. It was noted that patients taking cannabis regularly (not less than once per day) were more likely to experience beneficial effects with regards to dyskinesia (Venderová et al., 2004).

5.8 Contraindications of Cannabis Use in Treating Motor Symptoms

Many studies have shown marijuana to be ineffective in treating motor symptoms. For instance, Carroll and colleagues showed that ingested marijuana was ineffective in treating both Parkinsonian symptoms and LID (Carroll et al., 2004). The drug Cannador (which contains unequal parts CBD and Δ9-THC) was proven to be ineffective in the treatment of LID and other motor symptoms (Carroll et al., 2004). Cannabis did not affect PD induced tremors (Stampanoni Bassi et al., 2017). Standardized methods, including the UPDRS score, were used to assess motor fluctuations and LID after administration of the CB1 receptor antagonist rimonabant, which was shown to be ineffective against both (Mesnage et al., 2004). A review by the American Academy of Neurology concluded that cannabis is ineffective in treating dyskinesia (Koppel et al., 2014). The inconsistencies between research findings imply that the use of cannabis to treat the motor symptoms of PD should be investigated more thoroughly.

6 Cannabinoids and the Non-Motor Manifestations of PD

6.1 Effect on Sleep

It has been established that PD sufferers experience problems with sleep (Murillo-Rodriguez, Pastrana-Trejo, Salas-Crisóstomo & De-la-Cruz, 2017). This can be attributed to 2 factors, dopamine-related therapy (especially dopamine agonists), as well as the direct effects of the disease, which result in alterations in the sleep-wake cycle. Patients most commonly complain of fragmented sleep, disproportionate tiredness and parasomnia (Factor, McAlarney, Sanchez-Ramos & Weiner, 1990). Patients with PD experience a disturbance in rapid eye movement (REM) sleep, with lack of reduction of normal muscle atonia, thus they physically act out their dreams. This disorder is known as REM sleep behaviour disorder (RBD) (Lin & Chen, 2018).

Moreover, PD patients are prone to experience nightmares, resulting in them flailing their limbs and shouting in their sleep (Murillo-Rodriguez et al., 2017). CBD is the main cannabinoid associated with curing sleep disorders in PD. It helps treat behaviours related to RBD without causing any undesired effects. In four unrelated cases of PD with RBD, CBD improved RBD-related behaviours, and these symptoms returned on cessation of treatment (Chagas et al., 2014). These findings suggest that cannabis can improve the quality of life of PD patients by ameliorating their sleep.

6.2 Effect on Depression

Depression is present in 40% of PD patients. However, its cause is unclear (Barrero et al., 2005; Marsh, 2013). It may be a result of neurodegeneration in certain parts of the brain, or it may occur secondary to the symptoms of the disease as they impair the quality of life (Barrero et al., 2005). While there is a correlation between the severity of symptoms and occurrence of depression, depression is more common in PD than any other neurological disorder, which implies that the neurological changes that occur in this disease play a role in the occurrence of depression (Tandberg, 1996). Alterations in neurotransmitter signalling are associated with depression. For example, dopamine depletion plays a role in the development of depression, since it leads to anhedonia, lack of motivation and apathy (Cummings, 1992). Changes in CB1 receptor density may play a role in the development of depression as they interact with the monoamine systems. By activating CB1 receptors, Δ9-THC inhibits the synaptic release of dopamine, serotonin, GABA and norepinephrine, and its influence on dopaminergic and serotonergic function could be the cause of depression in cannabis users (Musty & Kaback, 1995).

Moreover, the limbic system of rats shows high mRNA levels of CB1 receptor genes, which is implicative of the association of CB1 receptors with emotions (Matsuda, Bonner & Lolait, 1993). There is a correlation between the occurrence of depression and expansion (of less than sixteen repeats) on one of the alleles of the gene for CB1. There is a highly polymorphic triplet sequence (AAT) on the CNR1 gene that is repeated varying amounts of times in different patients. If the gene is longer (i.e. the number of AAT repeats is higher), the likelihood of depression in PD patients decreases because it is less likely that the gene will be transcribed (Barrero et al., 2005). Several studies have shown that moderate marijuana use has anti-depressive effects. For example, activating CB1 receptors via Δ9-THC, increased serotonergic function and had anti-depressive effects (Bambico, Hat-

tan, Garant & Gobbi, 2012) in rats. The anti-depressive effect of Δ9-THC is CB1 receptor-mediated, as it ceases when the CB1 receptor antagonist rimonabant is administered (Navarro et al., 1997). Preventing the degradation of AEA also results in anti-depressive effects and enhances the activity of serotonin and norepinephrine in the midbrain (Gobbi et al., 2005). A study on the effects of cannabis on PD symptoms showed that 91.5% of subjects described their emotional state as depressed before cannabis treatment, with most of these patients reporting an improvement in mood following cannabis use (Balash et al., 2017).

6.3 Effect on Pain

The ECB system is expressed in various areas of the brain and spinal cord that modulate pain (Calignano, Rana, Giuffrida & Piomelli, 1998). AEA interacts with CB1 receptors in these areas to modulate pain pathways (Calignano et al., 1998). Pain is very commonly experienced in PD patients (Dworkin et al., 2003). Many analgesics have been used over the years, including gabapentin and opioids, but these have been associated with many adverse effects (Dworkin et al., 2003). Pain in PD has been linked to the presence of a single nucleotide polymorphism (SNP) in the gene encoding the FAAH enzyme (Greenbaum et al., 2012). Patients with PD experienced pain relief 30 minutes after smoking marijuana, which may be attributed to the feelings of well-being induced by the drug, as well as to its psychoactive effects (Lotan et al., 2014). In one study, 114 patients with PD (or Parkinson-plus syndromes) who experienced pain were treated with various analgesics. It was concluded that non-steroidal anti-inflammatory drugs (NSAIDs) were the most effective in treating pain (78% effective), and these were followed closely by medical marijuana (77% effective) (Yust-Katz, Hershkovitz, Gurevich & Djaldetti, 2017).

6.4 Neuroprotective Effect

Phyto-CBs prevented damage to neurons in models of PD due to their antioxidative properties and their ability to alter glial cell signalling (Lastres-Becker et al., 2005). CBD is both an antioxidant and an anti-inflammatory agent, which points to its ability to offer neuroprotection in PD patients. CBD can cross the blood-brain barrier and exert its effects on the brain. It was found that high concentrations of CBD are not toxic to PC12 treated cells, thus demonstrating its low toxicity, in fact CBD improved the viability of MPP+ treated PC12 cells, its effect being most successful at low doses (Santos et al., 2015). CBD prevents the death of dopaminergic neurons, thus aiding dopaminergic function in rat models of PD (Lastres-Becker et al., 2005; C. García et al., 2011). CBD attenuates reactive oxygen species and prevents the increase in expression of NADPH oxidase 1 and 4 (NOX1 and NOX4) (Pan et al., 2008). The superoxide produced by NOX enzymes is involved in the demise of dopaminergic cells in PD animal models (Hernandes, Café-Mendes & Britto, 2013). CBD decreases the formation of inducible nitric oxide synthase (iNOS) (Pan et al., 2008) and enhances the production of Cu^{2+}, Zn-superoxide dismutase mRNA, which when transcribed, prevents the formation of reactive oxygen species (García-Arencibia et al., 2007).

Cannabinoids can act as anti-inflammatory agents by preventing activation of microglia (which are found at higher levels in the SNc of patients with PD) and inhibiting the release of toxic cytokines (Sayd et al., 2014). Therefore, cannabinoids indirectly prevent dopaminergic cell death by counteracting neuroinflammation, one of the factors that contributes to their death (Clark & Kadodek, 2016). Cannabinoids also protect against neuroinflammation via reperfusion of the site of injury and by reversing vasoconstriction (Sagredo et al., 2007). CB2 receptor agonists (including cannabinoids) cause up-regulation of these receptors in microglia of the SNc, and also offer neuroprotection to dopaminergic neurons by shielding them from the neuroinflammation caused by the same activated microglia (C. García et al., 2011). Brain slices from Wistar rats were oxygen and glucose deprived, after which they were treated with the cannabinoid CB1/CB2 receptor agonist WIN55212. This drug prevented cell damage, production of nitric oxide and release of glutamate, among other neurotoxic effects (Fernández-López et al., 2006). By activating CB1 receptors, Δ9-THC also acts as an anti-inflammatory agent (Fishbein-Kaminietsky, Gafni & Sarne, 2014). CBD has been combined with Δ9-THC in various drugs, one of which is Sativex, and together, they have been proven to have neuroprotective effects (Iuvone, Esposito, De Filippis, Scuderi & Steardo, 2009). Δ9-THC can augment the survival of dopaminergic cells and enhance the activity of PPARγ receptors (Zeissler, Hanemann, Zajicek & Carroll, 2012). Apart from having neuroprotective properties of its own, CBD helps reduce the side effects associated with Δ9-THC administration. In such drugs, CBD and THC interact with both CB1 and CB2 receptors (Sagredo et al., 2011).

Additionally, Δ9-THCV plays a role in neuroprotection (C. García et al., 2011). In the long term, it counteracts the loss of dopaminergic cells in the SNc, which occurs as a result of 6-OHDA treatment. It also prevents the activation of microglia. It is likely that both of these effects are caused by Δ(9)-THCV's antioxidant properties, rather than its action on CB2 receptors, since they were also observed on treatment with CBD. To further support this hypothesis, when CB2 receptor KO mice were treated with 6-OHDA, their loss of dopaminergic cells was of the same degree as wild type mice, imply-

Table 1: An overview of the action of different cannabinoids on motor and non-motor PD symptoms.

Effect of cannabinoids on motor impairments			
Δ9-THC (> 50 ng/ml of its metabolite 11-nor-Δ-9-THCOOH detected in urine)	Humans	Improvement of bradykinesia and rigidity	Venderová, Ružička, Vořísek and Višňovský (2004)
Smoked cannabis (inhaled amount: 0.5 g)	Humans	Improvement of tremor, rigidity, bradykinesia. Little effect on posture	Lotan, Treves, Roditi and Djaldetti (2014)
Nabilone (dose not specified)	Humans and primate models	L-DOPA induced dyskinesia	More and Choi (2015)
Effect of cannabinoids on sleep			
CBD (75 mg/day and 300 mg/day were both effective)	Humans	REM sleep behaviour disorder related behaviour	Chagas et al. (2014)
Effect of cannabinoids on depression			
Δ9-THC (repeated administration of 1 mg/kg)	male adult Sprague–Dawley rats	Improved serotonergic function	Bambico, Hattan, Garant and Gobbi (2012)
Smoked cannabis (0.9 ± 0.5 g/day)	Humans	Improved mood	Balash et al. (2017)
Effect of cannabinoids on pain			
Cannabis (dose not specified)	Humans	Pain relief	Yust-Katz, Hershkovitz, Gurevich and Djaldetti (2017)
Smoked cannabis (inhaled amount: 0.5 g)	Humans	Pain relief	Lotan, Treves, Roditi and Djaldetti (2014)
Neuroprotective effect of cannabis			
CBD (1 µM dose was most effective)	MPP+ treated PC12 cells from rats	Antioxidant and anti-inflammatory	Santos et al. (2015)
Δ(9)-THCV (2 mg/kg^{-1} for 14 days)	6-hydroxydopamine-lesioned animals	Anti-oxidant	C. García et al. (2011)
WIN55212 (50 µM)	Glucose and oxygen deprived brain slices from Wistar rats	Prevents cell damage, production of nitric oxide and release of glutamate	Fernández-López et al. (2006)

ing that the presence of CB2 receptors did not affect cell loss (C. García et al., 2011). However, a conflicting study showed that CB2 receptors do play a role in neuroinflammation (Gómez-Gálvez et al., 2016). It was observed in cells of the SNc and striatum that had been treated with lipopolysaccharide (LPS), a neurotoxin used to mimic neuroinflammation and PD in animals, these receptors were up-regulated. The amount of activated microglia was also significantly higher in CB2 receptor deficient cells when compared to the wild type. Furthermore, when compared to the wild type animals, the loss of dopaminergic cells was more rapid, and occurred to a greater extent, in cells lacking CB2 receptors (Gómez-Gálvez et al., 2016). An overview of the effects of cannabinoids on the motor and non-motor symptoms of PD can be found in Table 1.

7 Conclusion

Medical marijuana has been suggested to have some potential benefits as a treatment for PD. From the literature reviewed here, CBs may be more effective against non-motor symptoms compared to motor symptoms, although meliorates LID. Marijuana has already been legalized in Israel for therapy of pain and tremor in people suffering from PD (Yust-Katz et al., 2017), and it is effective in treating comorbid sleep problems and de-

pression. It may act as a disease-modifying treatment by blocking/delaying disease progression due to oxidative stress and neuroinflammation, an effect that seems to be independent from CB receptors. Among the CBs, the most promising in this regard seems to be CBD.

Although there are contradictory studies which today do not support the therapeutic use of marijuana for different disorders such as PD, medicinal marijuana has been legalized in various areas of the world, including Malta. This step forward has been largely driven by the media, politics and the public, signifying the need for more preclinical/clinical research, including carefully planned clinical trial studies regarding the therapeutic effects of cannabis, in order to enable medical professionals to provide new effective cures for their patients.

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