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The *Cannabis sativa* herb contains over 100 phytocannabinoid (pCB) compounds and has been used for thousands of years for both recreational and medicinal purposes. In the past two decades, characterisation of the body's endogenous cannabinoid (CB) (endocannabinoid, eCB) system (ECS) has highlighted activation of central CB_1 receptors by the major pCB, Δ^9-tetrahydrocannabinol (Δ^9-THC) as the primary mediator of the psychoactive, hyperphagic and some of the potentially therapeutic properties of ingested cannabis. Whilst Δ^9-THC is the most prevalent and widely studied pCB, it is also the predominant psychotropic component of cannabis, a property that likely limits its widespread therapeutic use as an isolated agent. In this regard, research focus has recently widened to include other pCBs including cannabidiol (CBD), cannabigerol (CBG), Δ^9tetrahydrocannabivarin (Δ^9-THCV) and cannabidivarin (CBDV), some of which show potential as therapeutic agents in preclinical models of CNS disease. Moreover, it is becoming evident that these non-Δ^9-THC pCBs act at a wide range of pharmacological targets, not solely limited to CB receptors. Disorders that could be targeted include epilepsy, neurodegenerative diseases, affective disorders and the central modulation of feeding behaviour. Here, we review pCB effects in preclinical models of CNS disease and, where available, clinical trial data that support therapeutic effects. Such developments may soon yield the first non-Δ^9-THC pCB-based medicines.

Key words: cannabinoids, endocannabinoid system, CB_1 receptors, electrophysiology, epilepsy, feeding

Abbreviations

AD, Alzheimer's disease; AED, anti-epileptic drugs; AEA, arachidonylethanolamide; 2-AG, 2-arachidonylglycerol; CBC, cannabichromene; CBD, cannabidiol; CBDV, cannabidivarin; CB, cannabinoid; CBG, cannabigerol; CBN, cannibinol; DAGLα, diacylglycerol lipase α; eCB, endocannabinoid; FAAH, fatty acid amide hydrolase; FST, forced swim test; GPCR, G-protein-coupled receptor; HD, Huntington's disease; 6-OHDA, 6-hydroxydopamine; iNOS, inducible nitric oxide synthase; IN, interneuron; LPS, lipopolysaccharide; MES, maximal electroshock; MAGL, monoacyl glycerol lipase; MS, multiple sclerosis; NO, nitric oxide; NRS, numerical rating scale; PD, Parkinson's disease; pCB, phytocannabinoid; PC, Purkinje cell; rCBF, regional cerebral blood flow; SAD, seasonal affective disorder; SCE, standardised cannabis extract; SPST, stressful public-speaking test; TST, tail suspension test; Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^9-THCV, Δ^9tetrahydrocannabivarin; TRP, transient receptor potential; TH+, tyrosine hydroxylase positive.

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1. Introduction

This review focuses on the emerging potential of phytocannabinoids (pCBs) to act as novel therapeutic agents in CNS disorders, in particular, as assessed by the use of preclinical *in vivo* animal models of CNS disease and available clinical trial data. Cannabis has been used medicinally and recreationally for thousands of years with early documentation of medicinal use in Chinese pharmacopoeias (Li & Lin, 1974) and the Indian *Atharva Veda* which accords cannabis status as one of five sacred plants (Touw, 1981). Early texts on herbal medicines were summarized by Dioscorides in ~60 A.D. and by Galen, who wrote of cannabis in the 2nd century A.D. in his *De facultatibus alimentorum, "The leaves of this plant cure flatus – some people squeeze the fresh (seeds) for use in ear-aches. I believe that it is used in chronic pains*". Cannabis appeared in the 1788 New England Dispensatory, which retained large elements of Dioscorides herbal pharmacopoeia. Work of the 19th century Irish physician, William O'Shaughnessy, introduced medicinal use of cannabis to the UK (O'Shaughnessy, 1840), benefiting from the ascribed analgesic, anti-inflammatory, anti-emetic and anti-convulsant properties of the plant. However, medicinal use of cannabis fell out of favour in the early 20th century, largely due to concerns about psychoactivity and effects on behaviour, motor co-ordination and memory and learning; such concerns lead to cannabis being removed from the British Pharmacopoeia in 1932 (Ashton, 2001; Kalant, 2001; Robson, 2001). However, it was still possible for UK physicians to prescribe cannabis for specific medicinal uses up to 1973, until prohibition by the Misuse of Drugs Regulation; in the current iteration of this Act (1985), cannabis is classified in Schedule 1, meaning that therapeutic use is effectively prohibited (Moffat, 2002).

Despite these restrictions, interest in the pharmacology and potential therapeutic use of pCBs was engendered by the isolation of Δ^9-THC and the subsequent discovery of other pCBs (Gaoni & Mechoulam, 1971; Mechoulam, 2005). Thereafter, the development of synthetic CB receptor ligands, such as Pfizer's CP55,940 in the 1980s, led to the identification of specific Δ^9-THC binding sites in the human CNS (Herkenham *et al*., 1990) and the identification and cloning of the first CB receptor, CB_1 (Matsuda *et al*., 1990). These findings contributed to the discovery of the endocannabinoid (eCB) system (ECS) (a term introduced by Di Marzo & Fontana, 1995), which comprises the cannabinoid (CB) receptors, eCBs as their endogenous

ligands and the proteins responsible for eCB synthesis and degradation. Shortly thereafter, a second, principally peripheral, cannabinoid CB_2 receptor was identified in 1993 (Munro *et al*., 1993). Around the same time, arachidonic acid-derived, endogenous CB receptor ligands were identified, with the discovery of arachidonylethanolamide (AEA; Devane *et al*., 1992) and 2-arachidonylglycerol (2-AG) (Mechoulam *et al*., 1995; Sugiura *et al*., 1995). The first eCB degrading enzyme to be cloned was fatty acid amide hydrolase (FAAH; Cravatt *et al*., 1996), with a number of further degradation and synthetic enzymes being identified shortly afterwards (Patricelli & Cravatt, 2001); these enzymes have become a major target for therapeutic manipulation (Di Marzo, 2008, 2009). The discovery and characterisation of the ECS subserved a resurgence of interest in the pharmacological effects of the individual pCBs (Izzo *et al*., 2009; Pertwee, 2008).

Despite the therapeutic potential afforded by the discovery of the ECS, licensed pCB-based medicines have largely been restricted to the use of Δ^9-THC in a subset of chronically ill patients. Synthetically produced Δ^9-THC and its analogues are used clinically as dronabinol and nabilone, both used for attenuation of cancer chemotherapy-induced nausea and vomiting and appetite stimulation in HIV/AIDS patients. The widespread use of Δ^9-THC is limited by psychoactivity and the associated abuse potential. Δ^9-THC is a partial agonist at CB_1 receptors whilst, by contrast, the anti-obesity agent, rimonabant, was the first clinically licensed CB_1 receptor antagonist. However, as a result of psychiatric side effects (depression and suicidality) reported following usage of higher doses (Christensen *et al*., 2007), rimonabant sales were suspended in 2008. Sativex (an approximately 1:1 mixture of Δ^9-THC:CBD) is the first medicine derived from whole cannabis plant extracts to be licensed (at present in the UK, Canada, Spain, Germany, Denmark and New Zealand); specifically, to treat pain and spasticity in multiple sclerosis (MS) patients (Barnes, 2006; Perras, 2005). Most pertinently, the introduction of Sativex provided a precedent for the licensed therapeutic use of pCBs, a theme that will be further investigated here. The combination of CBD and Δ^9-THC in Sativex is considered to reduce unwanted effects of Δ^9-THC (Russo & Guy, 2006), most likely by CBD inhibiting the metabolism of Δ^9-THC to the more psychoactive 11-OH-Δ^9-THC (Bornheim & Grillo, 1998), and there is evidence that CBD can oppose Δ^9-THC effects *in vivo* (Malone *et al*., 2009; Vann *et al*., 2008). Thus, Sativex is an important development as it reduces Δ^9-THC

central actions to produce a drug which is more tolerable and less prone to abuse (Schoedel *et al*., 2011). In this regard, it is also possible that Δ^9-THC efficacy could be enhanced by 'entourage' effects of other pCBs present in the Δ^9-THC and CBD extracts of which Sativex is comprised (Russo, 2011). Overall, the investigation of alternative, non-Δ^9-THC pCBs which lack psychotropic effects, but retain pharmacological activity, and the elucidation of their mechanisms of action has increasingly become a focus of the pharmaceutical industry and their potential to combat CNS disease is the major focus of this review.

2. Synthesis and production of phytocannabinoids

pCBs are lipid-soluble chemicals present in the resin secreted from trichomes that are abundantly produced by female plants of the *Cannabis sativa* herb. It is worth highlighting that pCBs are not so named because they share a common pharmacological target site or mechanism of action to eCBs and synthetic CBs, but due to their shared chemical structure. Within the plant, pCBs are synthesised from fatty acid precursors via a series of transferase and synthase enzymes (Figure 1). The two major pCBs, Δ^9-THC and CBD, are derived from a common synthetic precursor, cannabigerol (CBG). From a pharmacochemical perspective, whilst Δ^9-THC and CBD have pentyl side chains, major homologues are Δ^9-tetrahydrocannabivarin (Δ^9-THCV) and cannabidivarin (CBDV) respectively, with propyl side-chains, derived from cannabigerovarin (CBGV). As discussed below, despite only small differences in chemical structure, these compounds appear to exhibit markedly different pharmacological properties. Other pCBs, such as cannabinol (CBN), are considered to be oxidation products. All pCBs are uniquely found in cannabis, with the total number of identified pCBs currently reported as over 100 (together with over 500 non-cannabinoid constituents; Elsohly & Slade, 2005; Mehmedic *et al*., 2010). The plant can be genetically manipulated to alter the relative ratios of the pCBs produced. Whilst this exploitable feature has been capitalised upon by the recreational drug market as a means to increase Δ^9-THC yields, it is only more recently that the approach has been successfully used to develop a legitimate medicinal product. Thus, it is possible to use solely horticultural techniques to produce cloned plants ('chemovars') which are uniformly enriched in different, specific pCBs (de Meijer *et al*., 2003). Analogous to pharmaceutical synthesis of drug material, these processes follow FDA

botanical guidelines (Food and Drug Administration, 2004) to transform a raw material into a botanical drug substance as an active pharmaceutical ingredient, which can then be formulated into a botanical drug product, such as the standardised cannabis extracts (SCEs) used in Sativex. Importantly, modulation of the ratio of specific pCBs in different SCEs may not only offer therapeutic potential dependent on the nature of the target disease, but also provide a viable intellectual property model to justify pharmaceutical industry development of cannabis-based medicines.

3. Phytocannabinoid molecular targets and mechanisms of actions

3.1. The endocannabinoid system (ECS)

The detailed characterisation of the ECS, including the molecular determination of CB receptors and the metabolic pathways and actions of eCBs, initially provided a useful framework to discuss pCB actions. CB receptor activity can be modulated directly by ligand binding, or indirectly, via modulation of eCB levels (for example by enzyme inhibition). CB_1 and CB_2 receptors are seven-transmembrane spanning proteins of the rhodopsin G-protein-coupled receptor (GPCR) family A, sharing 44% sequence identity overall with 68% identity in their transmembrane domains (Munro *et al*., 1993; Pertwee *et al*., 2010). The pertussis toxin-sensitive nature of CB receptor-induced adenylyl cyclase inhibition suggested a predominant coupling to inhibitory $G\alpha_{i/o}$ subunits (Felder *et al*., 1993). Within the CNS, CB_1 receptors are largely localized to presynaptic terminals, particularly in the cerebral cortex, hippocampus, cerebellum and basal ganglia, with little evidence of postsynaptic expression (Herkenham *et al*., 1990; Tsou *et al*., 1998). Activation of presynaptic CB_1 receptors, via the retrograde release of eCBs produced by postsynaptic cells following periods of sustained excitation (Alger & Kim, 2011), causes a inhibition of neurotransmitter release and dynamically modulates both excitatory and inhibitory neuronal activity in the CNS (Chevaleyre *et al*., 2006; Ma *et al*., 2008; Guggenhuber *et al*., 2010). Recent studies have identified CB_2 protein and mRNA at sites in the CNS (Van Sickle *et al*., 2005; Onaivi *et al*., 2006). CB_2 receptors are expressed in the CNS on astrocytes, microglia and cerebromicrovascular endothelial cells (Golech *et al*., 2004; Nunez *et al*., 2004; Rivers & Ashton, 2010) and such expression could play a role in pathogenesis and treatment of

conditions involving neuroinflammation and neurodegeneration (Arevalo-Martin *et al*., 2008; Cabral & Griffin-Thomas, 2009).

It is becoming apparent that pCBs exhibit a considerable range of affinities for the CB_1 receptor (Figure 2; Kreitzer & Stella, 2009; Pertwee, 2008; Pertwee *et al*., 2010). Δ^9-THC is believed to exert the majority of its actions in the CNS as a partial agonist at CB_1 receptors (Howlett, 2002). Amongst other pCBs, Δ^9-THCV is one of the few compounds known to exert direct and relatively potent effects at CB receptors, leading to its description as a CB_1 antagonist (although with evidence of CB_1 agonist properties at higher doses (>10 mg/kg *in vivo*)) and, also, a potent CB_2 receptor partial agonist (Thomas *et al*., 2005; Dennis *et al*., 2008; Ma *et al*., 2008; Pertwee, 2008; Bolognini *et al*., 2010). Interestingly, CBD shows only low CB receptor binding affinity (Bisogno *et al*., 2001; Pertwee, 2008; Jones *et al*., 2010; Figure 2), but has been shown to antagonise the action of synthetic CB ligands at CB_1 and CB_2 receptors (Pertwee *et al*., 2002; Thomas *et al*., 2007). In general, current knowledge for actions of other pCBs at CB receptors remains incomplete; however, CBG has been reported to exhibit only low CB receptor potency (Figure 2; Cascio *et al*., 2010), but antagonises the effects of CB_1 ligands in [^{35}S]GTPγS binding assays (Cascio *et al*., 2010). Moreover, recent pharmacological evidence has shown that the CB_1 receptor contains an allosteric binding site (Price *et al*., 2005; Horswill *et al*., 2007) and the allosteric CB_1 receptor antagonist, PSNCBAM-1 exerts agonist-dependent effects on inhibitory synaptic transmission in the CNS (Wang *et al*., 2011). The identification of an allosteric CB_1 site promises to drive the characterisation and development of novel probes and drug candidates, although any potential for pCBs to act via such sites is not known as yet. Overall, whilst these studies demonstrate that selected pCBs (i.e. Δ^9-THC and Δ^9-THCV) exert effects via direct interaction with CB receptors, other pCBs thus far investigated exhibit an alternative, but potentially therapeutically exploitable, pharmacology (Izzo *et al*., 2009).

More recent evidence has revealed that pCBs can exert effects via modulation of eCB tone in the CNS. The principal targets so far identified are the 2-AG biosynthetic enzyme, diacylglycerol lipase α (DAGLα), and the catabolic enzymes, FAAH and monoacyl glycerol lipase (MAGL), predominantly responsible for AEA and 2-AG hydrolysis, respectively (Di Marzo *et al*., 2005). A number of prominent non-Δ^9-THC pCBs show micromolar potency as ECS enzyme inhibitors *in vitro*: CBDV inhibits DAGLα, CBD inhibits FAAH whilst CBG and

cannabichromene (CBC) inhibit MAGL (Watanabe *et al*., 1996; Rakhshan *et al*., 2000; Bisogno *et al*., 2001; De Petrocellis *et al*., 2011). In a related fashion, micromolar concentrations of CBG, CBC, CBDV and CBN all inhibit cellular uptake of AEA (De Petrocellis *et al*., 2011). Whilst the functional effects of pCBs on eCB tone and their pharmacological relevance remain to be fully determined, this evidence suggests that pCB effects in the CNS are not limited to components of the ECS and such distinctions are discussed below.

3.2. Non-CB receptors and ion channel targets of pCBs

In addition to effects on the ECS, evidence arising from pharmacological experiments in recombinant cell lines and CB receptor knock-out animals strongly supports pCB actions at alternative, non-CB receptor sites. Orphan GPCRs, most notably GPR55 and GPR119, have been identified as potential novel CB receptors on the basis of affinity for some CB ligands (Pertwee, 2007; Ross, 2009; Pertwee *et al*., 2010). However, it is not yet clear whether GPR55 is a *bona fide* CB receptor, as it possesses low sequence homology to CB_1 and CB_2 and the endogenous phospholipid, lysophosphatidylinositol, also has affinity for the receptor (Nevalainen & Irving, 2010; Sharir & Abood, 2010). There are, thus far, limited reports of pCB activity at GPR55; for example, Δ^9-THC has a weak agonist effect, whilst reports of CBD as a GPR55 antagonist appear to be largely assay-dependent (Pertwee *et al*., 2010).

An interesting emerging concept is that pCBs can also activate non-CB metabotropic GPCRs. In particular, CBD has been widely reported to act as a 5-HT_{1A} agonist (Russo *et al*., 2005; Magen *et al*., 2010; Ledgerwood *et al*., 2011) and also to have actions sensitive to adenosine A2A receptor antagonists (Magen *et al*., 2009). Another recent study has shown that CBG is an agonist at α2-adrenoceptors and an antagonist at 5-HT_{1A} receptors (Cascio *et al*., 2010). It is also becoming clear that pCBs have the potential to affect neuronal excitability via the modulation of ligand-gated and voltage-gated ion channels (Pertwee, 2008; Pertwee *et al*., 2010). In particular, recent studies have highlighted the effects of a number of pCBs, including CBD, CBG, CBC and CBN, at different transient receptor potential (TRP) (ligand-gated non-selective cation) channels. CBD has been widely reported to activate TRPV1 and TRPV2 channels (Costa *et al*., 2004; De Petrocellis *et al*., 2008; Qin *et al*., 2008); interestingly, TRPV1 co-localises with CB_1 receptors in mouse brain (Cristino *et al*., 2006). More recently, CBG,

CBGV and Δ^9-THCV have also been shown to activate TRPV1 channels (De Petrocellis *et al*., 2011). Similarly, Δ^9-THC, CBD, CBGV, CBG, Δ^9-THCV and CBDV have all been shown to activate rat TRPV2 channels (De Petrocellis *et al*., 2011). pCB effects at TRPV1 and TRPV2 channels typically manifest at low micromolar concentrations, which does question, but not exclude, their pharmacological relevance; for example, reported actions included channel desensitization, akin to the proposed therapeutic action for agonists such as capsaicin. CBD, CBC, and CBN are more potent (nanomolar concentration) agonists at rat TRPA1 channels and also desensitise the channel (De Petrocellis *et al*., 2011). CBD, CBG, CBN, Δ^9-THCV, CBDV and CBGV (at low micromolar concentrations) all also act as antagonists at rat TRPM8 channels (De Petrocellis *et al*., 2011). CBD has also been demonstrated to act at ligand-gated receptors, being a putative allosteric inhibitor of 5-HT_{3A} receptors (Yang *et al*., 2010) and an allosteric and direct activator of inhibitory glycine receptors (Ahrens *et al*., 2009; Foadi *et al*., 2010). There is growing evidence that synthetic CBs and eCBs can modulate voltage-dependent Ca^{2+}, K^+ and Na^+ channels (Demuth &Molleman, 2006; Oz, 2006; Pertwee *et al*., 2010); at present, evidence for similar pCB actions at ion channels is limited. However, Δ^9-THC and CBD have recently been shown to inhibit $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ (T-type) Ca^{2+} channels (Ross *et al*., 2008). Therefore, both ligand-gated and voltage-dependent ion channels may be targeted by pCBs and it will be important to augment such studies using *in vitro* electrophysiology to determine the functional effects of pCBs on neuronal excitability and whether such effects are seen at pharmacologically relevant concentrations.

3.3 Neuroprotection and CNS immune function

pCBs are known to protect neurons from neurotoxic stimuli or neurodegeneration via a range of properties which may include ligand action at CB receptors, innate antioxidant properties and effects on the CNS immune system. Δ^9-THC has been shown to possess CB_1-dependent neuroprotective effects in excitotoxicity assays *in vitro* (Abood *et al*., 2001; Gilbert *et al*., 2007) and i*n vivo* (Chen & Buck, 2000; van der Stelt *et al*., 2001; El-Remessy *et al*., 2003; Zani *et al*., 2007). However, several studies have highlighted CB receptor-independent mechanisms by which Δ^9-THC and other pCBs can protect neurons. Most clearly described is the antioxidant capacity of pCBs. A study in 1998 first highlighted the CB_1 receptor-independent antioxidant

properties of Δ^9-THC and CBD (Hampson *et al*., 1998), demonstrating their ability to protect rat cortical neurons from glutamate receptor-mediated excitotoxicity, which is known to be mediated by reactive oxygen species. El-Remessy *et al*. (2003) showed that both Δ^9-THC and CBD protected rat retinal neurons against NMDA-induced neurotoxicity *in vivo*, decreasing levels of peroyxnitrite and associated oxidative stress-related compounds.

pCBs are also able to modulate immune cells and the production of immune factors in the CNS in experimentally-induced models of neurodegenerative disorders. The primary immune cells in the CNS are microglia which provide support to neural cells; in neurodegenerative diseases, microglia are co-localised to sites of neuronal death (Ramirez *et al*., 2005; Lull & Block, 2010). Agonism of CB_2 receptors on microglia attenuates their further activation (Carrier *et al*., 2004; Kreitzer & Stella, 2009; Stella, 2010), limiting ability of microglia to release pro-inflammatory agents including tumour necrosis factor α and nitric oxide (NO) (Ehrhart *et al*., 2005; Ramirez *et al*., 2005). Correspondingly, the agonist properties of both Δ^9-THC and Δ^9-THCV at CB_2 receptors have been implicated in neuroprotection *in vivo* (Tourino *et al*., 2010; Garcia *et al*., 2011). CBD has also been shown to be anti-inflammatory by limiting ATP-induced increases in intracellular Ca^{2+} levels and NO production in cultured microglial cells (Martin-Moreno *et al*., 2011). An anti-inflammatory effect of CBD was also observed in lipopolysaccharide (LPS)-injected mice due to inhibition of adenosine uptake (Carrier *et al*., 2006); a similar effect was seen *in vitro* and in rat retina insulted by LPS (Liou *et al*., 2008). Production of pro-inflammatory cytokines by LPS–stimulated cultured microglial cells was inhibited by CBD via a decreased activity of NF-κB, but increased activation of STAT3 (Kozela *et al*., 2010). Additionally, CBD decreased inducible nitric oxide synthase (iNOS) expression and TNFα levels in a mouse model of LPS-induced inflammation (Ruiz-Valdepenas *et al*., 2011).

In summary, it is clear that pCBs exhibit a range of apparently neuromodulatory, neuroprotective, anti-oxidant and anti-inflammatory properties, including effects on biochemical pathways that could complement their effects on receptors, ion channels and enzymes to achieve an overall therapeutic aim. The utility of such effects is discussed hereafter in an examination of pCB effects in animal models of CNS disease and human clinical trials.

4. Effects of phytocannabinoids in CNS disorder, disease and dysfunction

4.1 Phytocannabinoids in the treatment of epilepsy and hyperexcitability disorders

4.1.1.Historical background

Cannabis has played a historical role in the treatment of hyperexcitability disorders, a prominent example being epilepsy, where the first evidence of therapeutic use was attributed to the Arabic scholar al-Mayusi in 1100AD (Lozano, 2001), although additional evidence to support such use can be found in both Ayurvedic and Islamic medicine (Russo, 2005; Russo 2007). Cannabis use was again noted in the 15th century, when the historian Ibn al-Badri wrote that when "*the epileptic son of the caliph's chamberlain*" was treated with cannabis "*it cured him completely, but he became an addict who could not for a moment be without the drug*" (Mechoulam, 1986), a predictable consequence given the chronic, progressive nature of epilepsy. Thereafter, it was not until after William O'Shaughnessy successfully treated seizures in an infant using cannabis tincture (O'Shaughnessy, 1840) that further reports emerged describing attempts to use cannabis to treat seizures (McMeens, 1856, 1860; Reynolds, 1868).

In the 1970s, effects of several common cannabis constituents on seizure states were further examined using the maximal electroshock (MES) model (Karler *et al*., 1973; Karler *et al*., 1974b; Turkanis *et al*., 1974). These early studies revealed an order of potency of Δ^9-THC>CBD>CBN although, interestingly, the authors asserted that CBD had the greatest protective index, comparable to the, then widely used, anticonvulsant phenobarbital (for review, see Karler & Turkanis, 1981; Karler & Turkanis, 1976). These studies supported a number of small-scale human trials, individual case studies and surveys that investigated herbal cannabis and isolated pCB use for seizure control (Table 1). Whilst these studies stimulated a limited number of pre-clinical investigations (Chiu *et al*., 1975; Karler *et al*., 1974a; Smiley *et al*., 1976; Thomson & Turkanis, 1973; Turkanis *et al*., 1977; Turkanis & Karler, 1975; Turkanis & Karler, 1981a; Turkanis & Karler, 1987; Turkanis *et al*., 1991; Turkanis *et al*., 1979), the complex nature of epilepsy and the diverse model- and species-specific effects of cannabis (and individual pCBs) rendered the elucidation of mechanisms of action difficult, particularly in the case of Δ^9-THC, upon which attention had been largely focussed (Martin & Consroe, 1978; Consroe & Fish, 1980).

The renewed interest in potential therapeutic applications of pCBs included investigations using *in vitro* models of epileptiform activity (Wilkinson *et al*., 2003; Whalley *et al*., 2004) and, subsequently, *in vivo* models of seizure. Δ^9-THC clearly affects seizure states and susceptibility in preclinical models (Lutz, 2004; Boggan *et al*., 1973) via well-known effects at central CB_1 receptors (Shen & Thayer, 1999). However, Δ^9-THC (and other CB_1 agonists) often exhibit contradictory pro- and anti-convulsant effects in clinical cases (Table 1) and preclinical models (Karler & Turkanis, 1980; Turkanis & Karler, 1981b; Turkanis & Karler, 1982; Consroe & Mechoulam, 1987; Wallace *et al*., 2001; Fish *et al*., 1983). Together with psychotropic side effects, such contradictory effects likely limit or prohibit Δ^9-THC's widespread therapeutic use as an isolated agent. However, many surveys continue to report medicinal cannabis use for the control of seizures, which lends credence to an overall conclusion that the presence of Δ^9-THC in SCEs *per se* does not necessarily represent a *de facto* pro-convulsant risk. Moreover, in some clinical cases (Table 1), Δ^9-THC at higher doses can be an effective anticonvulsant, but is limited by extensive psychoactive side-effects. Overall, whilst the variability of Δ^9-THC's effects may represent a limiting factor, growing evidence supports attenuation of undesirable Δ^9-THC effects by pCB- and non-pCB components of cannabis (Russo, 2011), so improving its therapeutic index and legitimising the case-by-case use of Δ^9-THC-based medicines (e.g. 'medical marijuana') against seizures, as is currently the case in Canada and some US states.

4.1.2 Δ^9-tetrahydrocannabivarin (Δ^9-THCV) in hyperexcitability

Δ^9-THCV has demonstrated interesting potential for use in the treatment of hyperexcitability states. Following identification and characterisation of Δ^9-THCV as a CB_1 receptor antagonist (Thomas *et al*., 2005; Dennis *et al*., 2008), the increase of inhibitory synaptic transmission in cerebellar brain slices represented the first description of functional Δ^9-THCV effects in the CNS (Ma *et al*., 2008). In the latter study, Δ^9-THCV (5-58 μM) significantly increased GABAergic transmission at interneuron-Purkinje cell (IN-PC) synapses in patch clamp electrophysiological recording; complementary use of multi-electrode array (MEA) recording demonstrated that Δ^9-THCV significantly reduced spontaneous unit and multi-unit PC spike firing (Figure 3; Ma *et al*., 2008). Δ^9-THCV modulated the effects of the CB agonist

WIN55,212-2 and Δ^9-THCV actions were abolished by the $GABA_AR$ antagonist, bicuculline. Overall, these data were consistent with Δ^9-THCV antagonising CB_1 receptors at IN-PC presynapses to increase inhibitory neurotransmission (either via a blockade of eCB action or by attenuation of constitutive CB_1 activity) leading to a reduction in PC excitation. The ability of Δ^9-THCV to modulate PC output contrasts with the well-known adverse (partial) agonist effects of Δ^9-THC, which induces deficits in motor coordination *in vivo* (DeSanty & Dar, 2001a; DeSanty & Dar, 2001b; Patel & Hillard, 2001), a reported effect of cannabis intoxication. From the perspective of hyperexcitability states, the effects of Δ^9-THCV in increasing inhibition in the cerebellum is consistent with a desirable pharmacological profile for use in spinocerebellar ataxias, a progressive and presently pharmacologically untreatable group of hyperexcitability disorders (Paulson, 2009), although pre-clinical *in vivo* animal studies in this specific therapeutic area have yet to be undertaken.

More recently, Δ^9-THCV was reported to exhibit *in vitro* anti-epileptiform and *in vivo* anticonvulsant properties (Hill *et al.*, 2010a). In this study, Δ^9-THCV (>20 μM) significantly reduced burst complex incidence and the amplitude and frequency of paroxysmal depolarizing shifts (PDSs) induced by use of Mg^{2+}-free media (which activates excitatory glutamatergic NMDA receptors) in piriform cortical brain slices; Δ^9-THCV also inhibited the propagation of this epileptiform activity. This investigation also showed that pre-incubation of piriform cortical slices with 10 μM Δ^9-THCV significantly reduced neuronal excitability in response to Mg^{2+}-free media, consistent with the hypothesis that exposure to Δ^9-THCV may be prophylactic in preventing hyperexcitability. In the pentylenetetrazole model of acute generalised seizures, Δ^9-THCV (0.25 mg/kg) significantly reduced seizure incidence, although failing to affect other commonly employed seizure measures (Hill *et al*., 2010a). It has been shown recently that *in vivo* seizure states may be disrupted as a result of a CB_1 agonist-mediated desynchronisation of pathological neuronal firing (Mason & Cheer, 2009), similar desynchronisation could also hold true for CB_1 antagonist-mediated blockade of eCB tone; such a hypothesis is consistent with known Δ^9-THCV effects upon the propagation of epileptiform activity (Hill *et al.*, 2010a).

Overall, although the concept of presynaptic CB_1 receptor-mediated inhibition of excitatory neurotransmitter release being consistent with anti-epileptiform effects is intuitively

clear (Lutz, 2004), a mechanism underlying anticonvulsant Δ^9-THCV effects, alongside other confirmatory and contradictory reports of synthetic CB_1 antagonist effects in seizure models (Echegoyen *et al*., 2009; Kozan *et al*., 2009), is not immediately apparent. However, when a preferential CB receptor ligand effect is considered, such as that described above for inhibitory IN-PC synapses in the cerebellum (Ma *et al.*, 2008) or excitatory terminals in the hippocampus (Monory *et al*., 2006), it becomes clear that effects on neuronal excitability obtained via CB_1 modulation are likely to be highly dependent upon the sub-population of neurons (i.e. inhibitory or excitatory) preferentially affected (Lutz, 2004).

4.1.3 Cannabidiol (CBD) in hyperexcitability

CBD remains the only isolated, non-Δ^9-THC pCB to have been investigated for anticonvulsant effects in human subjects to date (Table 1). As early as 1977, CBD effects upon seizure states in animals were investigated using MES and audiogenic seizures and compared with those of standard anti-epileptic drugs (AEDs) including phenytoin, phenobarbital, carbamazepine, and ethosuximide (Consroe & Wolkin, 1977a). CBD (>100 mg/kg) administered alone was an effective anticonvulsant in both seizure models, but had differential effects when co-administered with standard AEDs, enhancing the anticonvulsant effects of phenytoin or phenobarbital, but diminishing the effects of chlordiazepoxide, clonazepam, trimethadione or ethosuximide (Consroe & Wolkin, 1977a; Consroe & Wolkin, 1977b). A potential advantage of CBD is that, unlike Δ^9-THC, no evidence of contradictory central excitatory or pro-convulsant effects exists (Chiu *et al.*, 1979). In electrically kindled limbic seizures in rats, CBD (0.3-3 mg/kg) raised epileptic after-discharge threshold in a manner consistent with the known effects of phenytoin in this model, but, in common with the effects of ethosuximide, also decreased after-discharge amplitude, duration and propagation (Turkanis *et al.*, 1979). It is notable that, compared to phenytoin and ethosuximide, the authors concluded that "*CBD was the most efficacious of the drugs tested against limbic ADs* [after-discharges] *and convulsions"*. CBD had a selective depressant effect upon evoked cortico-limbic responsiveness in non-epileptic states (Turkanis & Karler, 1981a). CBD (60 mg/kg) had no discernible effect in rats rendered chronically epileptic by cortical implantation of cobalt, which manifests as partial seizures with secondary generalisation (Colasanti *et al.*, 1982), whilst Δ^9-THC was found to exert a short

term (~1 day) anticonvulsant effect. It is however noteworthy that cobalt-induced seizures share many common features with human absence seizures (Loscher, 1997) and, as such, have little in common with seizure models in which CBD exerts a significant anticonvulsant effect. Such model-specific effects were also exemplified using a battery of acute models of seizures induced by agents that included MES, 3-mercaptoproprionic acid, picrotoxin, isonicotinic acid hydrazine, bicuculline, pentylenetetrazole and strychnine (Consroe *et al*., 1982). Here, CBD (50-400 mg/kg with most notable effects occurring at >100 mg/kg) was equally effective in the MES and all GABA inhibition-based models, but entirely ineffective against strychnine-induced convulsions.

More recently, CBD effects upon chemically-induced epileptiform activity in acute hippocampal brain slices have been described (Jones *et al.*, 2010). Here, CBD significantly reduced measures of spontaneous epileptiform activity induced either by use of Mg^{2+}-free media, or by the application of the K^+ channel blocker, 4-aminopyridine. In the Mg^{2+}-free model, CBD (100 μM) decreased epileptiform local field potential burst amplitude and duration. In the 4-aminopyridine model, CBD (100 μM) decreased burst amplitude in CA1 only, burst duration in CA3 and dentate gyrus, and burst frequency in all regions. The same report also recapitulated the previous investigation of CBD effects upon pentylenetetrazole-induced, acute, generalised seizures (Consroe *et al.*, 1982) and found that CBD (100 mg/kg) significantly decreased mortality and the incidence of the most severe seizure states. Finally, in this study, CBD was shown to exhibit only low affinity for CB_1 receptors in radioligand binding studies and no agonist activity in GTPγS binding assays, supporting a CB_1 receptor independent mechanism of anticonvulsant action (Jones *et al*., 2010; see also Figure 2).

Taken together, CBD exhibits the most reliable anticonvulsant effects of currently tested pCBs. Moreover, in contrast to clinically used anticonvulsants, CBD exhibits no neurotoxic or motor side-effects as assessed by standard rotarod tests (Consroe *et al*., 1981; Martin *et al*., 1987). Overall, recent data more fully supports the proposal for CBD potential in the treatment of grand mal, cortical focal, partial, but not absence seizures. In this regard, CBD exhibits a potential useful polypharmacology that may benefit modulation of neuronal excitability (Figure 4). In addition to the epilepsy-specific actions described above, CBD has been shown to reduce intracellular Ca^{2+} levels in hippocampal neurons under conditions modelling increased

excitability (Ryan *et al*., 2009). Such actions occur via an inhibitory action on mitochondria Ca^{2+} stores and are consistent with CBD possessing further useful actions to reduce hyperexcitability in the CNS.

4.1.4 Summary

Overall, Δ^9-THC effects in hyperexcitability disorders can be unpredictable and, based on the extant evidence, effects are likely to be specific to the disorder and the individual which is, given Δ^9-THC's psychoactive effects and the idiopathic and/or cryptogenic natures of most epilepsies, unsurprising. This, together with notable side effects, limits Δ^9-THC's widespread therapeutic use, although sufficient case studies have reported apparent benefit and so prevent the drawing of a single definitive conclusion applicable to all epilepsies. We have used complementary *in vitro* electrophysiological techniques to provide the first descriptions of non-Δ^9-THC pCB effects in hyperexcitability in the CNS; in particular, effects of Δ^9-THCV and CBD have been translated into preclinical *in vivo* seizure models and shown to possess therapeutic potential. Whilst Δ^9-THCV shows some promise in this regard, its clinical utility may be limited to pathophysiological conditions associated with CB_1 receptors preferentially located on inhibitory synapses. By contrast, there is clearly compelling evidence to support further investigation of CBD effects in human hyperexcitability states, either as an adjunct or standalone treatment. More broadly, whilst Δ^9-THCV and CBD's effects in seizure models cast new light upon the potential therapeutic use of cannabis constituents for the treatment of hyperexcitability disorders, it is notable that they still represent a minority of the pCBs present in cannabis. Consequently, further studies are required to assess whether other, as yet uninvestigated, pCBs modulate seizure activity from both the perspective of AED development and risks associated with cannabis use (Wilkinson *et al.*, 2003; Hill *et al.*, 2010b). In the near future, it will be important to extend investigations in disease models to fully determine their potential as therapeutic agents in their own right or their use as a structural basis for rational drug development, and then progress into clinical trials.

4.2 Phytocannabinoids in the treatment of CNS neurodegenerative diseases

4.2.1 Historical background

Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) are characterised by a progressive loss of viable, functional neurons within one or more regions of the CNS, leading to specific deficits that increase in severity as the disorder progresses. A strong neuroinflammatory response is also observed in AD and PD (Lee *et al*., 2010; Qian *et al*., 2010) and is characterised by activation of microglia and the release of inflammatory agents. This inflammatory response is now itself considered a significant pathological cause of neurodegeneration. Additionally, the autoimmune CNS disease MS is also understood to have a neurodegenerative element crucial to the pathology of the disease which worsens in parallel with the development of symptoms (Stadelmann *et al*., 2011). Thus, although AD, PD and MS have distinct aetiologies, they all exhibit both neurodegeneration and inflammation. As described above, cannabis has been used for many thousands of years in the treatment of a wide range of disorders and illnesses; the first historical uses of cannabis in a wide range of neurological disorders are comprehensively discussed in Russo (2007). Neuroprotective effects of cannabis have been suggested as early as ~1200 A.D. in India (Shou-Zhong, 1997) and more recently in the West when treating dementia (Reynolds, 1868). Prolonged use of an Indian hemp preparation was also reported to *"quiet the tremor for a time"* for a patient with Parkinson's in 1888 by Sir William Gowers (Gowers, 1888). More recently, a survey sent to PD sufferers treated at the Prague Movement Disorder Centre reported a benefit of cannabis in nearly half of respondents (Venderova *et al*., 2004). These reports, alloyed with the anti-inflammatory, antioxidant and immunomodulatory properties of several pCBs (Section 3.3) have led to preclinical research in animal models of neurodegenerative diseases and, in some cases, limited human trials in AD, HD and PD, which are outlined below.

Whilst a significant clinical benefit of pCBs for many neurodegenerative disorders has yet to fully manifest, the link between cannabis and the relief of MS symptoms, primarily spasticity, has a richer history. The first reference to the muscle relaxant properties of cannabis may have been as early as the 9th century A.D. (Russo, 2007), with further reports by O'Shaughnessy in the 19th century (O'Shaughnessy, 1840). Small-scale human studies into the beneficial effect of cannabis and Δ^9-THC on MS symptoms between 1983 and 2002 (for review see Rog, 2010), as well as anecdotal reports of benefits and a report from the British Medical Association (1997), prompted the British government to call for a large-scale clinical

investigation of the effects of cannabis on MS (House of Lords, 1998). Additionally, changes in the ECS, particularly CB_1 receptor expression, have been shown to occur in human and experimental Parkinsonism (Silverdale *et al*., 2001; Hurley *et al*., 2003; Walsh *et al*., 2010) and Huntington's disease (HD) (Blazquez *et al*., 2011), suggesting CB_1 receptors may be a target for drug interventions in these diseases. In the following section we discuss the extant, largely clinical, data regarding the effect of SCEs on MS symptoms and associated preclinical research. Following this, the preclinical research on pCB effects in animal models of AD, PD and HD is summarised, as are the limited number of relevant human studies.

4.2.2 Phytocannabinoids in multiple sclerosis (MS)

MS is a chronic, progressive disease that is most frequently diagnosed in young adults. The majority of patients experience acute attacks followed by months or even years of remission, with attacks becoming progressively more severe in later life (Compston & Coles, 2008). The pathological basis of MS is the formation of inflammatory, demyelinating lesions in the CNS with resultant axonal loss, neuronal death and sclerotic plaques result (for review, see Stadelmann *et al*., 2011). Preclinical research from animal models of MS has suggested a potential role for pCBs in the attenuation of inflammation and the protection of neurons at risk of damage. As early as 1989, Δ^9-THC was reported to delay or prevent signs of symptom onset in the experimental allergic encephalomyelitis model of MS in mice, as well as increasing survival rates and decreasing neuroinflammation (Lyman *et al*., 1989). Recent work has indicated that CB_1 and CB_2 agonists, including Δ^9-THC, can limit symptoms, relapses, axonal loss and neuroinflammation in rodent models of MS (Arevalo-Martin *et al*., 2003; Croxford & Miller, 2003; Docagne *et al*., 2007; Maresz *et al*., 2007; Hasseldam & Johansen, 2010). Δ^9-THC (10 mg/kg) has also been reported to control spasticity in the chronic relapsing experimental allergic encephalomyelitis mouse model of MS via a CB_1-dependent mechanism (Baker *et al*., 2000).

Clinical investigation of SCEs in the treatment of MS symptoms have focussed on extracts with Δ^9-THC and CBD as their primary active ingredients; other pCB/plant matter is minimised at <10%. The reader is directed to two additional reviews by Lakhan & Rowland (2009) and Rog (2010) for a more detailed description of these studies. The cannabinoids in MS study (CAMS) investigated the effects of dronabinol (Marinol, a synthetic Δ^9-THC) and

Cannador (2.5:1.25mg Δ^9-THC:CBD SCE delivered in capsule). In a randomised, large-scale, placebo-controlled trial, neither dronabinol nor SCE (maximum Δ^9-THC dose 25mg/day) significant affected objective (Ashworth Scale) measures of spasticity, but strong positive outcomes were observed for both drugs against control as assessed by patient-reported measures of spasticity and pain (Zajicek *et al*., 2003). A one-year follow up in which patients remained on their treatment suggested that the patient-reported benefit is maintained (Zajicek *et al*., 2005). The pattern of strong significant improvements in patient-reported measures of spasticity combined with changes in objective spasticity measures in favour of the SCE, but not significantly so, is common for SCE clinical studies; however, a significant improvement in Ashworth Scale scores was reported by Vaney *et al*. (2004) after treatment of 57 patients for two weeks with a Δ^9-THC/CBD SCE (maximum dose 30.8 mg/10.8 mg Δ^9-THC/CBD per day).

Sativex (2.7:2.5 mg/100 μl spray marketed as Nabiximols) is delivered as an oromucosal spray, the benefit of which is a faster plateau of plasma concentrations compared to the oral route (GW Pharmaceuticals, 2001). Sativex is now licenced in a number of countries for adjunctive treatment of spasticity in MS, as well as for neuropathic pain in Canada. Wade and co-workers measured the effect of Sativex (<120 mg/day) on a variety of MS symptoms, and found that patient-reported (visual analogue scale) spasticity scores were significantly lowered by Sativex (Wade *et al*., 2004). A long-term open label extension found that Sativex maintained this beneficial effect (Wade *et al*., 2006). Collin *et al*. (2007) also reported significant improvements in spasticity as measured by a patient-reported daily numerical rating scale (NRS) score of spasticity, the primary endpoint. Secondary outcomes (including Ashworth Scale outcomes) were non-significantly in favour of Sativex (up to 48 doses/day Sativex). Most recently, a large-scale trial used an initial four-week single-blind Sativex regimen to identify a patient population that responded well to Sativex (maximum 12 sprays/day; Novotna *et al*., 2011). Around 40% of patients had spasticity NRS results that were improved by ≥20% in the first four weeks; these responders were randomised into a double-blind, placebo-controlled study (12 weeks), and Sativex was shown to significantly improved spasticity NRS scores and several other secondary outcomes, including spasm frequency and sleep disturbances.

Sativex, CBD and dronabinol were each found to be effective in treating MS-related and neuropathic pain in a recent meta-analysis (Iskedjian *et al*., 2007). Additionally, one double-

blind, randomised placebo-controlled five-week trial reported a significant effect of Sativex in alleviating MS-related pain (maximum 48 sprays/day; Rog *et al*., 2005). This effect was maintained without signs of tolerance in an open-label, uncontrolled two year extension that recruited participants from the previous trial (Rog *et al*., 2007). Further investigation into whether pCBs can alter the progression of MS in addition to effectively ameliorating symptoms could provide further justification for cannabis-based treatments for this disease, such investigations are currently in progress in the CUPID (Cannabinoid Use in Progressive Inflammatory Brain Disease) long-term (three year) study in which 493 MS patients are randomised to a placebo or Δ^9-THC treatment group (Clinical Neurology Research Group, 2009).

4.2.3 Phytocannabinoids in Alzheimer's disease (AD)

AD is the most common form of dementia, with age being a significant risk factor. AD is associated with the formation of neurofibrillary tangles, senile plaques and cortical atrophy (Perl, 2010). At present, there is limited preclinical data regarding the effects of pCBs in animal models of AD. A single *in vitro* study has suggested that Δ^9-THC competitively inhibits acetylcholinesterase (Eubanks *et al*., 2006), a therapeutic strategy that is approved to treat mild to moderate AD (Ellis, 2005). Iuvone and colleagues have shown that CBD ($\geq$0.1 μM) decreases levels of β-amyloid-associated reactive oxygen species and lipid peroxidation in PC12 cells and *in vivo* (Iuvone *et al*., 2004). Extending these studies, Esposito *et al*. (2006) demonstrated that CBD ($\geq$1 μM) attenuated a β-amyloid-induced increase in iNOS, also decreasing levels of p38 MAP kinase and NF-κB, both of which are involved in the response to oxidative stress. The same group showed that CBD (2.5 and 10 mg/kg) attenuated the β-amyloid inflammatory response *in vivo* by limiting iNOS and IL-1β expression (Esposito *et al*., 2007). More recently, Martin-Moreno *et al*. (2011) have shown that CBD (20 mg/kg) can limit microglial activation in *in vitro* and *in vivo* models of AD.

In line with limited preclinical data available at present, there is no published data describing clinical effects of pCBs on human AD (Krishnan *et al*., 2009), with the exception of a single, small (six subject), open-label, non-placebo controlled study which reported that synthetic Δ^9-THC (dronabinol; 2.5 mg/day) alleviates night-time agitation in patients with AD or vascular

dementia (Walther *et al*., 2006). Reports of CBD effects on *in vitro* and preclinical *in vivo* models of AD, allied with the high tolerability of CBD in humans, suggest that further investigation of therapeutic potential is merited in AD, particularly given that seizures are a common symptom of AD (Leppik & Birnbaum, 2010) that could also benefit from the anticonvulsant effects of CBD described previously.

4.2.4 Phytocannabinoids in Parkinson's disease (PD)

PD is primarily a movement disorder characterised by bradykinesia, tremor at rest and rigidity. The death of nigral dopaminergic neurons that innervate the striatum and modulate motor behaviour is responsible for these motor symptoms, resulting in a loss of tyrosine hydroxylase positive (TH+) neurons in the substantia nigra and reduced dopamine levels in the striatum. Neuropsychiatric symptoms are also common, as are sleep disturbances (for review, see Hindle, 2010). The effects of CB receptor ligands and pCBs on neuronal death, motor symptoms and inflammation have been widely investigated in preclinical animal models of Parkinsonism and there is evidence that pCBs can provide symptomatic relief and neuroprotection from experimentally-induced Parkinsonism.

Evidence for the efficacy of Δ^9-THC in ameliorating motor symptoms in PD models is mixed. Meschler *et al*. (2001) found that Δ^9-THC (>1 mg/kg) exacerbated of Parkinson-like bradykinesia induced by administration of MPTP, a toxin that kills dopaminergic neurons, into the substantia nigra in cynomolgus monkeys. By contrast, Δ^9-THC (~4mg/kg) caused improvement in both activity and hand-eye coordination in MPTP-treated marmosets (van Vliet *et al*., 2008). The contrasting results could be explained by the use of different monkey species, MPTP- and Δ^9-THC-dosing regimens, and clinical measures of Parkinsonism. In MPTP-treated marmosets, CB receptor agonism by the synthetic Δ^9-THC analogue (nabilone, ≥0.1mg/kg) has been reported to decrease L-DOPA-induced dyskinesias (Fox *et al*., 2002). Rats that received daily treatment with Δ^9-THC or CBD (both 3 mg/kg) for 2 weeks post-lesion had significantly higher levels of TH mRNA and dopamine ipsilateral to the lesion compared to vehicle-treated animals in the 6-hydroxydopamine (6-OHDA) model of PD (Lastres-Becker *et al*., 2005). This neuroprotective effect is likely to be CB_1-independent, due instead to the antioxidant capacity of pCBs (Garcia-Arencibia *et al*., 2007).

Δ^9-THCV has an attractive range of properties in relation to PD, Δ^9-THCV is likely to share the neuroprotective antioxidant properties possessed by other pCBs and can act as a CB_2 agonist *in vivo* (Bolognini *et al*., 2010; Pertwee, 2008), and can therefore affect microglial activation; additionally, Δ^9-THCV is a CB_1 antagonist, and the CB_1 antagonist SR141716A (0.1 mg/kg) has been shown to ameliorate motor symptoms in animal models of Parkinsonism (Gonzalez *et al*., 2006; Garcia-Arencibia *et al*., 2008; Kelsey *et al*., 2009). Acute administration of Δ^9-THCV (2 mg/kg) has recently been shown to improve motor performance in the 6-OHDA model of Parkinsonism in rat (Garcia *et al*., 2011); Δ^9-THCV increased striatal glutamate, but not dopamine, levels in a manner consistent with CB_1 antagonism. In the same study, chronic Δ^9-THCV (2 mg/kg) administration partially protected TH+ cells from 6-OHDA-induced death, attenuated microglial activation and also exerted a significant neuroprotective effect on nigral TH+ neurons in the LPS model of PD in mice. The LPS model exhibits greater CB_2 up-regulation than the 6-OHDA model; the effectiveness of a CB_2-specific agonist and the exacerbation Parkinsonism in mice lacking CB_2 receptors suggest Δ^9-THCV may be neuroprotective in a CB_2-dependent manner in this model (Garcia *et al*., 2011).

Investigation of the effects of cannabis and pCBs in human PD is limited. Small-scale human studies have investigated the effect of nabilone (Sieradzan *et al*., 2001) and Cannador (Carroll *et al*., 2004) on dyskinesias caused by the most common PD treatment, L-DOPA. Whilst nabilone (0.03 mg/kg) was reported to significantly improve dyskinesias in pilot trial in 7 patients as assessed by the Rush Dyskinesia Scale (Sieradzan *et al*., 2001), two patients withdrew due to adverse side-effects. Cannador (maximum Δ^9-THC dose of 0.17 mg/kg/day) had no effect on dyskinesia as assessed by several parameters, although it was well-tolerated, possibly due to an earlier dose escalation study to determine suitable dosages (Carroll *et al*., 2004). CBD alone ($\leq$400 mg/day) has been reported as effective in the treatment of PD-associated psychosis over four weeks of treatment in six consecutive patients presenting with three or more months history of psychosis (Zuardi *et al*., 2009), consistent with findings that CBD is an anti-psychotic (Zuardi *et al*., 2006). However, a study primarily concerned with dystonia found that CBD (>300 mg/day) aggravated Parkinsonism in two patients (Consroe *et al*., 1986). The Venderova *et al*. (2004) survey referred to previously represents the most promising finding regarding PD and human use of cannabis. A quarter of respondents reported cannabis (predominantly oral, not

smoked) use for PD symptom relief. Nearly half (45.9%) described a mild or substantial alleviation of symptoms above that provided by their prescribed treatment. Of these individuals, significant numbers reported improvements in resting tremor (30.6%) and bradykinesia (44.7%), 14.1% also reported alleviation of L-dopa-induced dyskinesia. 4.7% reported a worsening in symptoms (Venderova *et al*., 2004). This study, whilst a simple survey, indicates that further work is merited.

The use of pCBs in the treatment of various facets of preclinical experimentally-induced Parkinsonism appears promising. Specifically, although at an early stage of investigation, the combined properties of Δ^9-THCV (antioxidant, CB_1 receptor antagonist, CB_2 receptor agonist) hold promise in combating neurodegenerative, immunological and motor function symptoms of PD; the anti-inflammatory and antioxidant properties of CBD are also attractive. The current clinical evidence is very limited in scope, and therefore whilst findings are not uniformly positive, more extensive human studies are required to ascertain whether preclinical promise can be translated into treatments for this age-dependent, poorly-controlled disorder.

4.2.5 Phytocannabinoids in Huntington's disease (HD)

HD is a movement disorder that also causes cognitive and behavioural changes (for review, see Kumar *et al*., 2010). An autosomal dominant mutation of the Huntingtin protein is responsible for HD, causing neuronal death in the striatum and other areas of the brain, with spiny GABAergic neurons most affected (Gil & Rego, 2008). HD is also associated with a loss of CB_1 receptors (Blazquez *et al*., 2011). CBD and Δ^9-THC (both 5mg/kg) were neuroprotective in the 3-nitropropionic acid-induced striatal lesion HD model (Lastres-Becker *et al*., 2004; Sagredo *et al*., 2007); the effects of Δ^9-THC were most likely mediated by CB_1 receptors, whilst the effects of CBD were proposed to be due to antioxidant properties. However, daily treatment with Δ^9-THC (10 mg/kg over 8 weeks) reportedly had no effect on motor deterioration in a mouse transgenic model of HD (Dowie *et al*., 2010).

A small clinical trial following daily CBD (300-600 mg) treatment demonstrated an improvement in HD symptoms in 1 of 4 participants (Sandyk *et al*., 1989); however, a double-blind randomised placebo-controlled crossover trial in 15 HD patients showed no significant effect of CBD (10 mg/kg/day, 6 weeks) on chorea severity (Consroe *et al*., 1991). More recently,

a randomised, double-blind crossover placebo-controlled trial with 37 patients investigating the effects of the synthetic Δ^9-THC analogue nabilone (1-2 mg) showed no effect on the primary outcome (the Unified Huntington's Disease Rating Scale), but some evidence of improvement in chorea and neuropsychiatric outcomes (Curtis *et al*., 2009). As with other disorders, further clinical research is required into the effects of pCBs in HD to elucidate the potential benefits. The recent finding that loss of striatal CB_1 receptor expression may be an important factor in the pathogenesis of HD (Blazquez *et al*., 2011) indicates that the ECS system is a rational target for HD treatment, which may include pCB-based medicines.

4.2.6 Summary

The ability of a combination of Δ^9-THC and CBD to decrease symptoms associated with MS has led to the introduction of Sativex, the first licensed pCB drug. Moreover, there is increasing preclinical evidence that indicates pCBs may also be of benefit in treating the development of several neurodegenerative disorders; in particular, CBD's ability to modulate immune cell activity in the CNS and limit oxidative stress is promising and confers strong neuroprotective capacity. However, it should be noted that previously proposed, putative treatments for neurodegenerative diseases that exploit antioxidant and anti-inflammatory strategies have, in many cases, met with limited clinical success (Dumont & Beal, 2011; Whitton, 2010). Apart from the positive data gathered in the past decade on the effects of SCEs on MS symptoms, at present there is very little human data available on pCB effects in neurogdegenerative disorders. Thus, coordinated clinical trials investigating the effect of pCBs on both disease progression and symptom control for a range of neurodegenerative disorders are required to determine if and how pCBs can benefit patients with AD, HD and PD, all of which have a significant unmet clinical need. Encouragingly, most pCB-based treatments investigated to date, independent of the target disorder, appear to be well-tolerated, a promising sign for further clinical studies. Whilst most research has been performed on CBD, other pCBs share antioxidant capacity and may be more suited to specific diseases states. For example, Δ^9-THCV effects in models of PD appear to limit both neuronal cell death and the associated immune response whilst decreasing signs of bradykinesia.

4.3. Phytocannabinoids in affective disorders

In this section, we consider affective disorders; it is notable that pCBs also have effects in non-affective psychosis disorders, including schizophrenia (Hallak *et al*., 2011), however, such actions are not considered here.

4.3.1 Historical background

Cannabis has been used as a treatment for mood disorders for several thousand years, with well-documented use as a hypnotic and tranquilizer in the treatment of anxiety, mania, and hysteria (Mechoulam *et al*., 1970). Use of the plant continued into the early part of the 20th Century, where extracts have been used for their sedative and hypnotic properties to treat insomnia, melancholy, mania, and delirium (Russo & Guy, 2006). However, a decline and eventual cessation of cannabis use in psychiatry occurred over the last 100 years due to the development of new and more selective hypnotic and sedative drugs with well-characterised modes of action, alongside prohibition of use of the plant. However, the recent isolation and identification of pCBs with little or no psychoactivity is of particular relevance here and gives rise to the prospect of new therapeutic agents which may be used for the treatment of the affective disorders.

4.3.2 Phytocannabinoids in anxiety

Cannabis use has been associated with a high prevalence of anxiety; however, individual pCBs have been shown to possess anxiolytic properties (Crippa *et al*., 2009; Crippa *et al*., 2011) and thus use of specific pCBs (or selected combinations thereof) may hold as yet unexploited, therapeutic potential in the treatment of anxiety disorders.

There is evidence to suggest a significant comorbidity between cannabis use and prevalence of anxiety disorders. Reilly *et al*. (1998), using a structured interview in a rural area of Australia, found that 21% of long-term cannabis users reported high levels of anxiety, paranoia or depression. Similarly, Saban *et al*. (2010) investigating the relationship between substance misuse and psychopathology in high school students, reported a significant association between cannabis use and levels of anxiety. Furthermore, a recent study in Italian university students demonstrated a link between cannabis use and levels of anxiety which may, in turn, trigger risky and suicidal behaviour (Innamorati *et al*., 2008). In a study with 18-year-old New

Zealanders, it was reported that those who had smoked cannabis at least ten times between the ages of 15 and 16 had twice the prevalence of anxiety disorders compared to those who had never used the drug (Fergusson & Horwood, 1997). Likewise, in a study investigating emotion regulation and mental health problems, Dorard *et al*. (2008) found that more than half of the cannabis abusers reported comorbid diagnosis of CNS disorders, most commonly affecting mood and anxiety.

By contrast, it has been suggested that subjects with high levels of anxiety and patients with anxiety disorders use cannabis as a form of "self-medication" to treat symptoms. In support, an elegant analysis of the US National Comorbidity Survey showed that a large proportion of subjects developed anxiety disorders prior to the onset of their first symptoms of cannabis dependence, implying that the subjects were self-administering cannabis as an anxiolytic medication (Agosti *et al*., 2002). In line with this hypothesis, Buckner & Schmidt (2008) examined the temporal sequencing between the onset of seasonal affective disorder (SAD), alcohol misuse and cannabis dependence. Using a sample of participants from the Oregon Adolescent Depression Project, it was reported that SAD was an independent risk factor for the subsequent onset of cannabis dependence (Buckner & Schmidt, 2008). Overall, whilst cannabis may be anxiogenic in otherwise healthy cohorts, there are clear indications of anxiolytic effects in sufferers of anxiety disorders

The anxiolytic effects of CBD have been thoroughly investigated in preclinical models. The earliest reported study by Zuardi & Karniol (1983) showed that purified CBD (10 mg/kg) significantly decreased conditioned emotional responses to a stimulus in rats. Resstel & colleagues used a restraint stress paradigm in rats, which raises blood pressure and increases heart rate indicative of human anxiety behaviour, to demonstrate that CBD (1- 20 mg/kg) decreased acute autonomic responses (Resstel *et al*., 2009). Similarly, Guimaraes *et al*. (1990) showed that mice treated with 2.5-10 mg/kg (but not 20.0 mg/kg) CBD spent a greater amount of time in the open arm of an elevated plus maze, an effect similar to that produced by the standard anxiolytic agent diazepam. The anxiolytic actions of CBD have also been demonstrated in the mouse model of social defeat (Pistovcakova *et al*., 2006), the Vogel conflict test (Moreira *et al*., 2006), the conditioned fear paradigm (Resstel *et al*., 2006) and the contextual fear memory

extinction paradigm (Bitencourt *et al*., 2008). Taken together, the results from animal studies suggest that CBD has anxiolytic potential.

Preclinical data have led to a number of studies investigating possible anxiolytic actions in healthy and clinical human populations. An early study using healthy volunteers subjected to a stressful public-speaking test (SPST) showed that CBD (300 mg) reduced the volunteer's subjective anxiety to levels comparable with the standard anxiolytic, diazepam (Zuardi *et al*., 1993). A follow-up study by the same group (Crippa *et al*., 2004) used functional neuroimaging to demonstrate that CBD (400 mg) significantly decreased subjective anxiety; importantly, CBD also significant decreased regional cerebral blood flow (rCBF) in the left hippocampal and parahippocampal gyrus regions, indicative of an action on limbic and paralimbic brain areas. Later, Fusar-Poli *et al*. (2009) used functional magnetic resonance imaging to investigate neural correlates of the anxiolytic properties of CBD, demonstrating that CBD (600 mg) reduced amygdala, anterior cingulate cortical and posterior cingulate cortical activity in 15 healthy subjects subjected to a sequence of fearful facial stimuli. A recent study substantiated the role of CBD, whereby increases in anxiety induced by the SPST on subjects with SAD was reduced by CBD (600 mg) (Bergamaschi *et al*., 2011). In a clinical context, Crippa *et al*. (2011) investigated the effects of CBD treatment in 10 patients with generalised SAD; CBD (400 mg) significantly reduced subjective anxiety and led to reduced rCBF in left parahippocampal gyrus, hippocampus and inferior temporal gyrus, while increasing rCBF in right posterior cingulated gyrus. The authors suggest that CBD produces its anxiolytic actions due its effects on activity in limbic and paralimbic brain areas. To date, no studies have investigated the actions of other pCBs on anxiety, but it seems that CBD has promise as an anxiolytic agent. The description of both anxiogenic and anxiolytic actions of ingested cannabis also raise the possibility that individual pCBs have differential effects on anxiety; for example, anxiolytic CBD may oppose the anxiogenic effects of Δ^9-THC (Zuardi *et al*., 1982); such a description would fit well with the general concept that CBD can usefully ameliorate unwanted Δ^9-THC effects discussed previously.

4.3.3 Phytocannabinoids in depression

Elevation in mood and reduction in levels of stress following recreational cannabis use have been documented anecdotally for many years (Skolnick *et al*., 2001). Indeed, a recent internet survey comparing individuals with differing levels of marijuana use showed that those who used marijuana daily and those who used marijuana once per week or less reported less depressed mood and more positive affect than non-users (Denson & Earleywine, 2006). However, a review of the literature also reveals that cannabis ingestion is associated with an increased incidence of bipolar disorders and depression (Jarvis *et al*., 2008; van Rossum *et al*., 2009). As a result of these bi-directional effects research has largely focussed on understanding the role of the ECS in the pathogenesis and treatment of depression, rather than an investigation of the potential therapeutic actions of pCBs (for a recent review see Parolaro *et al*., 2010). Here, we will restrict our discussion to studies that have investigated the actions of individual pCBs in depressive syndromes.

The suggestion of a potential antidepressant role for Δ^9-THC is widespread. In the early 1980's the effects of Δ^9-THC (2.5 and 10 mg/kg delivered by paced smoking of herbal cigarettes) showed increases in relaxation and decreases in subjective ratings of anxiety, tension and depression (Ashton *et al*., 1981). In a clinical setting, significant antidepressant actions of Δ^9-THC treatment have been documented in patients suffering advanced cancer (Regelson *et al*., 1976), MS (Martyn *et al*., 1995; Svendsen *et al*., 2004) or chronic pain (Notcutt *et al*., 1997; Wade *et al*., 2003). However, as suggested above, evidence in support of a cannabis antidepressant action is equivocal. An early study by Kotin *et al*. (1973) reported that in 8 hospitalized patients with moderate to severe depression, Δ^9-THC administered for up to 7 days failed to exhibit any significant antidepressant response; however, the small sample size, limited study duration and relative severity of symptoms may hinder a firm conclusion from this study.

A comprehensive analysis of the potential antidepressant action of isolated pCBs has recently been reported by El-Alfy *et al*. (2010), where antidepressant actions of major pCBs were evaluated in the forced swim test (FST) model in mouse. Compounds that showed an anti-depressant action in the FST were additionally tested in the tail suspension test (TST). Both the FST and TST are standard preclinical tests used to measure the effect of antidepressant drugs. Classically, the results of these tests have been interpreted such that the time spent immobile is considered a behavioural correlate of negative mood. Treatment with 2.5 mg/kg Δ^9-THC (but not

1.25 or 5.0 mg/kg) produced a significant reduction in overall immobility time in both the FST and TST, consistent with an antidepressant action. Interestingly, similar reductions in time spent immobile in both the FST and TST were also seen with CBC. Here, 20 mg/kg CBC elicited decreased immobility time in the FST, whilst both 40 and 80 mg/kg CBC were effective in the TST. Finally, 200 mg/kg CBD decreased time spent immobile in the FST, but failed to show any further anti-depressant actions in the TST; treatment with Δ^8-THC, CBG or CBN failed to elicit any antidepressant-like actions (El-Alfy *et al*., 2010). Only one other study has investigated the actions of CBG to alleviate depression, CBG (40-80 mg/kg) produced significant reductions in the time spent immobile in the TST, with comparable effects to a known anti-depressant dose of imipramine (Musty & Deyo, 2006). Work by the same authors has also demonstrated a potential anti-depressant role for CBC (greatest activity seen at 40 mg/kg) using the TST (Deyo & Musty, 2003).

Of the non-Δ^9-THC pCBs, CBD appears to be the most thoroughly researched for its antidepressant actions. However, as highlighted by El-Alfy *et al*. (2010), results to-date have not always been consistent. Following the successful demonstration that CBD administration could reduce the behavioural consequences of restraint stress (Resstel *et al*., 2009), it was further shown that CBD (30, but not 3, 10 or 100 mg/kg doses) increased time spent swimming in the FST (Zanelati *et al*., 2010); interestingly, pre-treatment with a 5-HT_{1A} receptor antagonist blocked CBD action. Finally, in a small-scale human trial, 2 patients suffering bipolar affective disorder and experiencing a manic episode failed to show any improvement in symptoms in response to CBD treatment for 25 days (initial oral dose of 600 mg/day rising to 1200 mg/day), although this may reflect a differing aetiology underlying positive and negative symptoms in bipolar disorder (Zuardi *et al*., 2010).

4.3.4 Summary

These data suggest that, in the future, individual pCBs may have important therapeutic advantages over the ingested cannabis used in earlier studies in the treatment of affective disorders. At present, CBD is the most likely pCB to be translated into clinical practice due to its non-psychoactivity, safety and tolerability. However, long-term, double-blind, placebo-

controlled trials with subjects suffering from different affective disorders are still necessary and critical for this to be realised.

4.4 Phytocannabinoids and feeding-related disorders

In this section, we will explore the actions of the pCBs on food intake, a phenomenon that is intimately associated with modulation of feeding circuits in the hypothalamus by the eCB system, at present proposed to be principally due to an action on CB_1 receptors (Pagotto *et al*., 2006; Di Marzo *et al*., 2009). Whilst a detailed description of brain reward circuitry and interactions with the eCB system is beyond the scope of this article, several authors have reviewed these aspects in detail (Cota *et al*., 2003; Kirkham, 2008). It is clear that food intake may activate eCBs to stimulate reward pathways to engender further feeding behaviour. CB_1 receptor antagonists are well-known anti-obesity agents (Lee *et al*., 2009); however, obesity *per se* is not a solely CNS disease, rather, our discussion will be related to clinical conditions such as cachexia, anorexia and malnutrition, including the establishment of such conditions as a consequence of diseases such as AIDS, cancer and AD, diseases in which a disorder of appetite is a core feature.

4.4.1 Historical background

The appetite-stimulating, orexigenic properties of marijuana have been documented as far back as 300 A.D. (Chopra & Chopra, 1939). However, this seemingly well-substantiated phenomenon was previously only sparsely supported by empirical evidence, with few detailed human studies and even fewer well-controlled animal studies. In an early report, Hollister (1971) demonstrated that a single oral dose of marijuana (containing 0.35 mg/kg Δ^9-THC) significantly increased intake of milkshakes in normal, unfasted volunteers. Foltin and colleagues showed that volunteer subjects given marijuana cigarettes (1.84 % w/w Δ^9-THC) showed a markedly increase in food intake (1500 kcal), primarily attributable to an increase in snack food items (Foltin *et al*, 1986; Foltin *et al*., 1988).

In animals, the first comprehensive dose-response analysis of Δ^9-THC hyperphagia in rats was documented in the late 1990s (Williams *et al*., 1998); a range of Δ^9-THC doses were administered orally to pre-satiated rats with significant hyperphagia seen at doses $\geq$0.5 mg/kg Δ^9-THC. Subsequently, this hyperphagia was shown to be mediated by CB_1 receptors (Williams &

Kirkham, 2002b), and involved a marked reduction in latency to begin feeding (Williams & Kirkham, 2002a). Together these effects imply that the stimulation of feeding induced by Δ^9-THC may be linked to the appetitive phase of feeding, being associated with orienting an animal toward food and increasing the salience or reward value of food stimuli. The concept of cannabinoids influencing reward processes is well-established and has been supported by findings that blockade of CB_1 receptors by SR141716A reduced sensitivity to the rewarding effects of electrical brain stimulation (Arnold *et al*., 2001; Deroche-Gamonet *et al*., 2001) and blocked the acquisition of drug- or food induced place preferences (Chaperon *et al*., 1998). Conversely, stimulation of CB_1 receptors underlie the motivation to obtain and ingest palatable ingesta (Gallate & McGregor, 1999; Gallate *et al*., 1999; Higgs *et al*., 2003). Overall, current evidence suggests that animals work harder to obtain food after Δ^9-THC treatment, and eat earlier and more frequently when food is freely available.

At present, clinical interventions involving pCBs in syndromes affecting food consumption are dominated by use of Δ^9-THC and synthetic analogues. Cachexia is characterised by metabolic changes associated with a severe loss of appetite (McGrath, 2002) and is a common feature of the later stages of diseases such as AIDS and metastatic cancer (Cat & Coleman, 1994; Inui, 2002). Thus, treatments aimed at stimulating appetite by enhancing the attractiveness and enjoyment of food should be beneficial in these circumstances. Sacks and colleagues found that treatment with dronabinol (5 mg, three times daily) had little effect on food intake, but greatly attenuated the reduction in daily energy intake produced by chemotherapy (Sacks *et al.*, 1990). In the field of HIV-wasting syndrome, chronic daily dronabinol treatment (5-20 mg, orally for up to 20 weeks), caused a highly significant increase in appetite and mood ratings, with the majority of patients gaining weight over the course of treatment (Plasse *et al.*, 1991). Similarly, Beal *et al*. (1995) evaluated the long-term effects of Δ^9-THC or placebo in patients with AIDS-related appetite and weight loss; dronabinol (2.5 mg, twice per day over 42 days) administered to patients who had suffered progressive weight loss, experienced either stabilization of their body or a modest weight gain, accompanied by substantial increases in appetite.

Wasting and loss of appetite are also important features of ageing and associated conditions such as dementia (Morris & Volicer, 2001; Hickson, 2006). It is therefore possible

that the appetite-stimulating properties of cannabinoids may be a useful tool in attempting to maintain proper nutrition in these populations. Dronabinol (5 mg per day over 6 weeks) produced significant weight gain, but not increases in energy intake, in food-refusing dementia patients (Volicer, 1997). Finally, a possible target for the application of cannabinoids to stimulate appetite and overcome food refusal may be in the treatment of anorexia nervosa, a psychiatric condition exemplified by self-starvation. Dronabinol has been used to successfully manage appetite in elderly patients suffering from anorexia and significant weight loss (Wilson *et al*., 2007). By contrast, a study in 11 female patients with primary anorexia nervosa failed to show an effect of Δ^9-THC on daily changes in weight and caloric intake versus an active diazepam placebo (Gross *et al.*, 1983). However, it should be noted that the underlying psychopathology of anorexia is very complex, and involves significant psychological factors that are unrelated to any dysfunction in the normal physiological mechanisms controlling eating.

*4.4.2 Phytocannabinoid standardised cannabis extracts (SCEs) in feeding-**related disorders***

Despite the evidence of Δ^9-THC stimulatory effect on feeding and appetite detailed above, relatively few studies have investigated the contribution of non-Δ^9-THC pCBs to the feeding effects of cannabis. However, recent work has demonstrated that a range of pCBs may have significant effects on feeding patterns (reviewed in Farrimond *et al*., 2011). In an initial study, the effects of purified Δ^9-THC, synthetic Δ^9-THC and Δ^9-THC SCEs (which also contain an array of non-Δ^9-THC pCBs) were compared (Farrimond *et al*., 2010a). Importantly, all treatments were matched to a range of Δ^9-THC doses known to induce hyperphagia. Using standardised pre-feed paradigm (Williams *et al*., 1998), Δ^9-THC SCE showed significantly lower hyperphagia in comparison to the synthetic and purified Δ^9-THC doses; these data suggested that the combination of pCBs (and, potentially, non-pCB components) in the SCE attenuated the hyperphagic effects of Δ^9-THC. In a follow-up study (Farrimond *et al*, 2010b), SCEs containing concentrations of Δ^9-THC between two- and ten-fold lower than those previously demonstrated to induce hyperphagia, caused pre-satiated rats to significantly increase chow intake by reducing their latency to the first contact with food. These effects on feeding replicated those previously seen with much higher concentrations of pure Δ^9-THC (Williams *et al*., 1998; Farrimond *et al*., 2010a) and indicate that cannabis compounds other than Δ^9-THC may also have the ability to

stimulate appetite, effects that were concealed when a higher concentration of Δ^9-THC was present in the extract. It is clear from the data presented above (Farrimond *et al.*, 2010a; Farrimond *et al.*, 2010b) that the precise composition of an SCE is critical in determining the action on feeding, and that individual pCBs may antagonise the appetite-stimulating actions of Δ^9-THC, whilst others may have appetite-stimulating properties themselves. Finally here, the action of two SCEs, one of these containing 67% Δ^9-THC, the other a Δ^9-THC-free SCE have been investigated; all remaining pCBs in the SCE were kept constant (CBD: 0.3%; CBG: 1.7%; CBC: 1.6%; Δ^9-THCV: 0.9%; THCA: 0.3%; CBN: 1.5%; and sesame oil vehicle; total mixture dose range: 0.5 - 4.0 mg/kg) (personal communication, J Farrimond). Administration of both Δ^9-THC-free and 67%-Δ^9-THC SCEs induced highly significant dose-dependent increases in food consumption in the first hour after food was returned to the animals. This effect was attributed to highly significant reductions in the latency to the onset of feeding produced by both SCEs. However, some differences between the extracts were evident when considering other meal pattern parameters; most significantly, the 67% Δ^9-THC SCE significantly increase the duration of the first meal, whilst the Δ^9-THC-free SCE failed to induce any significant effect. This finding echoes those of previous studies (Farrimond *et al.*, 2010b), further implicating non-Δ^9-THC pCBs in the appetitive actions of feeding only.

Despite these promising findings, only one single clinical trial has been undertaken to investigate the effects of an SCE on appetite and feeding. Strasser *et al.* (2006) compared the effects of Cannador (2.5 mg THC and 1 mg CBD), Δ^9-THC (2.5 mg) and placebo on appetite and quality of life in patients with cancer-related anorexia-cachexia syndrome (CACS). Here, adult patients suffering significant weight loss were treated twice daily for 6 weeks with measures of appetite, mood, and nausea monitored daily. Results showed no significant differences between the three treatments, with increased appetite ratings of 73%, 58%, and 69% for patients receiving Cannador, THC, or placebo, respectively.

4.4.3 Individual phytocannabinoids in feeding-related disorders

Animal data presented to date strongly indicate that the non-Δ^9-THC pCBs present in the SCEs produce significantly effects on the appetitive, but not consummatory, aspects of feeding behavior. Thus, determination of the effects of individual pCBs are clearly warranted; however,

prior to 2009 there were few studies investigating the actions of individual non-Δ^9-THC pCB on feeding, with the majority of these studies being either unreplicated or even contradictory. In all cases, no detailed analyses of changes to feeding microstructure had been undertaken, which necessarily limits the interpretation of these findings. In 1976, Sofia and Knobloch examined the acute effects of CBN and CBD (both 50 mg/kg) on food and sucrose consumption. In this paradigm, animals were pre-trained to consume their total daily food intake during a 6 hour feeding period; water, 5% sucrose or 20% sucrose solutions were also available during this period. Both CBN and CBD significantly reduced food intake, effects which persisted for 4-5 days post-drug administration (Sofia & Knobloch, 1976). CBN and CBD produced similar reductions in sucrose intake, which returned to pre-baseline levels by day 3-4 post-drug administration. The authors interpreted these findings as suggestive that CBN and CBD produced a preference for sweet calories. It should also be noted that the Sofia & Knobloch (1976) study used doses of CBN and CBD between 200 and 1500 times greater than the concentrations of these compounds used in other studies that have suggested that non-Δ^9-THC pCB stimulate feeding (Farrimond *et al*., 2010a; Farrimond *et al.*, 2010b). Wiley *et al*. (2005) showed that CBD (3-100 mg/kg) failed to significantly alter food intake in mice; yet it should be noted that doses of 3 and 10 mg/kg CBD showed a non-significant trend towards an increase in intake suggesting that CBD may be worthy of further investigation. However, a recent study by Scopinho *et al*. (2011) further demonstrated that CBD (1, 10 or 20 mg/kg) failed to alter feeding and failed to replicate the non-significant trend towards an increase in feeding at low doses. CBD (2.5 and 5 mg/kg/day for 14 days) has been reported to produce significant decreases in body weight in rats, although no measures of food intake were taken (Ignatowska-Jankowska *et al*. 2011); interestingly, CBD action was sensitive to co-administration of the CB_2 receptor selective antagonist, AM630, suggesting a CB_2 receptor mechanism may be critical to the action of CBD on body weight.

In general, there is a broad literature implicating CB_1 receptor antagonists as potential anti-obesity agents; however, the recent failure of rimonabant has highlighted the need to develop safer alternatives (Lee *et al*., 2009; Izzo *et al*., 2009). In this regard, Riedel *et al*. (2009) have investigated the feeding effects of Δ^9-THCV (3, 10 and 30 mg/kg) and a Δ^9-THCV SCE (containing between 0.1 and 0.3 mg/kg Δ^9-THCV). All doses of Δ^9-THCV significantly reduced

food intake during the 12 h following treatment, whereas Δ^9-THCV SCEs did not affect consumption. This study confirms that purified Δ^9-THCV can reduce food intake in mice, which is worthy of further investigation. In particular, future work should investigate effects of purified Δ^9-THCV and Δ^9-THCV SCE using conditions which would be expected to maximise food intake (e.g. during the dark phase of the light:dark cycle or following periods of deprivation), thus ensuring high baseline food intake, maximizing the ability to detect any Δ^9-THCV-induced decreases in food intake.

4.4.4 Summary

The association between the effects of exogenous CBs and appetite gave a strong lead in suggesting possible physiological roles for the ECS in feeding-related diseases. Indeed, Δ^9-THC induces a degree of over-eating that far exceeds that produced by most other hyperphagic pharmacological manipulations. Crucially, the behavioural adjustments induced by exogenous CBs suggest that these compounds are involved in the processes which drive us to eat. Animals work harder to obtain food after CB_1 stimulation, and eat earlier and more frequently when food is freely available. CB_1 receptor agonists thus seem to actively *provoke* feeding, rather than merely prolonging eating that has been initiated through other mechanisms. More recent data has additionally shown a role for some non-Δ^9-THC pCBs in the stimulation of appetite, however, no studies have clearly delineated the individual pCB which may underlie these appetite-stimulating actions. Thus, further studies in animal models and humans are needed to confirm the ability of individual pCBs to alter food intake, and to clarify the mechanisms of action underlying these effects, such initiatives may lead to the development of novel therapeutic strategies for the treatment not only of feeding disorders themselves, but also disorders arising as a symptom of other CNS diseases.

5. Conclusions

The demonstration that *Cannabis sativa* contains numerous pCBs in addition to the major psychoactive Δ^9-THC component provides the impetus to support a solid body of preclinical studies focussing on therapeutic development of non-Δ^9-THC pCBs. Work in animal models of diseases is now being extended to an increasing number of clinical trials in human CNS

disease. The latter, in particular, has been fuelled by the introduction of the first SCE- and, by extension, pCB-, based medicine, Sativex. As well as providing a useful proof-of-concept, the introduction of Sativex may serve to lower the barriers to the perceived societal difficulties associated with cannabis-based medicines. In general, where Δ^9-THC has been shown to be an effective treatment in animal models or clinically, the adverse side effects of CB_1 agonism need to be weighed against the clinical benefit to patients; however, the combination of Δ^9-THC and CBD into an SCE yields a medicine that is well-tolerated in the clinic, suggesting that the presence of Δ^9-THC does not necessarily preclude the development of medicines suitable for widespread use.

Whilst, generally still in their infancy, clinical data for effects of individual (or mixed) non-Δ^9-THC pCBs may be usefully extended to trials for feeding-related disorders, neurodegenerative diseases, affective disorders and epilepsy, amongst others. This review has highlighted CBD, as a compound with a multi-modal mechanism of action, with clear therapeutic potential in a number of these areas, befitting its status as the second most prevalent pCB in cannabis (Lerner, 1963). In general, whilst it can be seen that large doses of CBD can be tolerated in humans, it is worth pointing out that formulation of CBD (or other pCBs), for example with lipid vehicles or dispersing surfactants, during potential drug development could substantially increase bioavailability. It is also apparent that other pCBs, such as Δ^9-THCV and CBG, may have a similar therapeutic potential, but that further preclinical work is needed to justify human trials. It is also clear that non-Δ^9-THC pCBs act at a wide range of molecular targets and may possess useful additional properties, such as anti-oxidant capacity, to support their pharmacological profile. A recurring issue is a pharmacological relevance of some of the pCB actions described herein; in this regard, pCBs typically exhibit functional responses with low micromolar potencies. A caveat here is that due to their high lipophilicity, for studies conducted in, for example, brain slice preparations, pCBs may partition into lipid membranes leading to underestimations of effective potency (discussed in Ma *et al*., 2008; see also Brown *et al*., 2004). Importantly, despite the relatively high concentrations required at some targets, pCBs such as Δ^9-THCV and CBD are known to penetrate the blood–brain barrier well, with no major toxicity, genotoxicity, or mutagenicity (Hill *et al*., 2010a; Jones *et al*., 2010). Based on measurements of CSF levels in rat, we have calculated that 100 mg/kg CBD doses i.p. reach

CNS concentrations of ~18 μM which suggests that low micromolar potencies can have functional relevance. In this regard, CBD doses as high as 1200 mg have been safely tolerated in human trials (Trembly & Sherman, 1990; see Table 1). Thus, the future of pCBs as safe and efficacious agents to combat CNS disease holds great pharmacological and therapeutic promise.

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Figure legends

Figure 1. Biosynthesis of major phytocannabinoids.

Figure 2. Effect of phytocannabinoids on SR141716A binding in mouse cerebellum membranes. Competition binding assays for phytocannabinoids in comparison to standard synthetic CB_1 ligands against 1 nM [^{3}H]SR141716A. B) Δ^9-THCV has micromolar affinity and CBD and CBG have millimolar affinity for CB_1 receptors.

Figure 3. Effect of Δ^9-THCV in mouse cerebellar brain slices. A) In patch clamp recording from IN-PC synapses, Δ^9-THCV(58 μM) increased frequency of miniature inhibitory postsynaptic currents and blocked agonist effects of WIN55,212-2 (WIN55; 5 μM). B) In MEA recording from cerebellar slices (i), WIN55 (5 μM)-induced increases in PC spike firing were significantly reversed by Δ^9-THCV (5-40 μM); * $p<0.05$ (Mann-Whitney U-test)

Figure 4. CBD has a multi-modal action. Scheme showing some identified molecular targets and potential modes of actions for CBD in central neurons.

Table1: Summary of human case studies and clinical trials employing cannabinoids or cannabis in which a pro- or anti-convulsant effect was observed. The limited nature of some sources occasionally render information regarding study design, dosage routes, compound purity and origin unavailable. Extant and pertinent information has been included below.

Report type	*Study drug*	*Pro- or anti-convulsant*	*Primary outcome*	*Reference*	*Notes*
Clinical trial	Δ^9-THC	Anti-convulsant	'*severe anticonvulsant resistant grand mal epilepsy controlled*' in 2/5 children; no change to 3/5 children.	Davis & Ramsey (1949)	Δ^9-THC administered to 5 institutionalised children, previously unresponsive to phenobarbital and phenytoin.
Case study	Smoked cannabis	Anti-convulsant	Full control of seizures	Consroe *et al.* (1975)	Isolated report of one adult using phenytoin and phenobarbital who only achieved full seizure control when also using smoked cannabis. Seizures returned when phenytoin and phenobarbital were withdrawn and only cannabis smoked.
Clinical trial	Oral CBD (≤300 mg per day)	Anti-convulsant	4/8 CBD treated patients with full seizure control, 1/8 improved markedly, 2/8 improved somewhat, 1/8 no improvement. In placebo-treated patients 1/8 showed a little improvement, 7/8 showed no change.	Carlini & Cunha (1981)	Small (15), adult patient cohort all with partial seizures with secondary generalisation that were unresponsive to conventional treatment; double blind study design employing CBD and placebo.
Clinical trial	Oral CBD (200-300 mg per day)	No change	No significant change to seizure incidence	Ames & Cridland (1986)	Findings only published in abstract form which yields limited information; 12 patients enrolled; CBD given as an adjunct to existing treatments.
Survey of cannabis use in patients admitted to hospital after first seizure	Cannabis	Anti-convulsant	The authors concluded that '*marijuana use* [is] *a protective factor for new-onset seizures*'	Ng *et al.*, (1990)	Survey of 308 patients admitted to hospital after first seizure compared to 294 control patients with no seizure; the results were criticised as '*weak*' by a 1999 US Institute of Medicine report '*Marijuana and medicine: Assessing the science base'* since *'the study did not include measures of health status prior to hospital admissions and differences in their health status might have influenced their drug use*'

Clinical trial	CBD (900-1200 mg per day for 10 months)	Anti-convulsant	'*seizure frequency was markedly reduced in the patient*'	Trembly & Sherman (1990)	Open label clinical trial; Results presented at conference and cited in: British Medical Association. Therapeutic uses of cannabis. Harwood Academic Publishers, Amsterdam, 1997; p51
Case studies	Cannabis	Anti-convulsant	Qualitative reports of successful seizure control with cannabis in three epilepsy patients	Grinspoon & Bakalar (1997)	
Case studies	Cannabis	Anti-convulsant	Qualitative reports of 11 patients successfully self-treating seizures with cannabis	Petro (1997)	Patients identified as applicants to the US Compassionate Use Investigational New Drug Programme to provide legal medical exemption from prosecution for cannabis possession and use.
Survey	Cannabis	Anti-convulsant	4% of patients supported by a medical marihuana programme reported use for seizure control	Corral (2001)	Survey population size: 77
Survey	Cannabis	Anti-convulsant	1% of clinical cannabis users in California reported use for seizure control	Gieringer (2001)	Survey population size: ~2500
Survey	Cannabis and Δ^9-THC	Anti-convulsant	1.4% of German medical users of cannabis and THC reported use for seizure control	Grotenhermen & Schnelle (2003)	Survey population size: 143
Case studies	Δ^9-THC	Anti-convulsant	'*Anticonvulsive action*'	Lorenz (2004)	0.04–0.12 mg/kg administered orally
Survey	Cannabis	Anti-convulsant	'*The majority of active users* [reported] *beneficial effects on seizures*'	Gross *et al.*, (2004)	Telephone survey of epilepsy patients
Survey	Cannabis	Anti-convulsant	Dutch Ministry of Health ordered (1999) monitoring of Δ^9-THC content of all legally supplied cannabis following reports of reduced seizure duration and incidence in cannabis users.	Pijlman *et al.* (2005)	
Clinical trial	Δ^9THC /CBD (Sativex)	Pro-convulsant	Four patients experienced '*first ever seizures'*	Wade *et al.* (2006)	Open label clinical trial in multiple sclerosis patients
Case study	Cannabis	Anti-convulsant	'*Marked improvement*' in seizure control following marijuana use	Mortati *et al.* (2007)	Adult cerebral palsy patient

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