# *Cannabis sativa and the endogenous cannabinoid system: therapeutic potential for appetite regulation*

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# *Cannabis sativa* and the endogenous cannabinoid system: therapeutic potential for appetite regulation

Short title: A therapeutic role for *C. sativa* in appetite regulation

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**Abstract**

The herb *Cannabis sativ*a (*C. sativa*) has been used in China and on the Indian subcontinent for thousands of years as a medicine. However, since it was brought to the U.K. and then the rest of the western world in the late 19$^{th}$ century, its use has been a source of controversy. Indeed, its psychotropic side effects are well reported but only relatively recently has scientific endeavour begun to find valuable uses for either the whole plant or its individual components. Here, we discuss evidence describing the endocannabinoid system, its endogenous and exogenous ligands and their varied effects on feeding cycles and meal patterns. Furthermore we also critically consider the mounting evidence which suggests non-$\Delta^9$tetrahydrocannabinol phytocannabinoids play a vital role in *C. sativa*-induced feeding pattern changes. Indeed, given the wide range of phytocannabinoids present in *C. sativa* and their equally wide range of intra-, inter- and extra-cellular mechanisms of action, we demonstrate that non-$\Delta^9$tetrahydrocannabinol phytocannabinoids retain an important and, as yet, untapped clinical potential.

**Keywords:** Feeding, appetite, phytocannabinoid, endocannabinoid, $\Delta^9$tetrahydrocannabinol

## Introduction

For ~10,000 years, *Cannabis sativa* (*C. sativa*) has been used medicinally and recreationally for its diverse pharmacological actions and psychotropic properties. Typical actions include sedation, analgesia, hypothermia, catalepsy and euphoria which are thought to arise mainly from cannabinoid type 1 receptor ($CB_1R$) activation (Childers *et al.*, 1998; Little *et al.*, 1988). These effects have typically been ascribed to the sixty (Howlett, 2002; Pertwee, 2008b) to eighty (Izzo *et al.*, 2009) phytocannabinoids (pCBs) present in *C. sativa*. In more recent times, *C. sativa* has been used less widely for medicinal purposes, due in part to the development of other therapeutic agents with better side effect profiles (e.g. Tramer *et al.*, 2001). Indeed, cannabinoid side-effects are known to include reduced motor coordination, tachycardia, transient memory loss, disruption to the sense of time, cognitive impairments, anxiety and a disruption of appetite regulation (Childers *et al.*, 1998; Dewey, 1986; Grant *et al.*, 2005; Hollister, 1986; Kirkham *et al.*, 2001b; Wilkinson *et al.*, 2003) (for a recent review on the safety of *C. sativa*-based therapy see Wang *et al.*, 2008). However, the relatively recent identification of the $CB_1R$ and cannabinoid type 2 ($CB_2R$) receptors, alongside the subsequent characterisation of the endocannabinoid (eCB) system (for a recent review see Maccarrone, 2009), has driven a resurgence of interest in the potential applications of cannabinoid-based therapy. Cannabinoid therapies have now been investigated for a wide range of neurological and neurodegenerative disorders (Glass, 2001; Pryce *et al.*, 2003), both in terms of treatment (for a review see Consroe, 1998) and/or symptom management (Davis *et al.*, 2007). One notable example is in the treatment of multiple sclerosis (Pryce *et al.*, 2005) and its symptoms (eg. spasticity), for

which a cannabis-based compound (Sativex®) has recently been licensed in the U.K., Spain and Canada by GW Pharmaceuticals.

'Cannabinoid' is a well-known term used to describe the compounds isolated from *C. Sativa*, the pCBs such as $\Delta^9$tetrahydrocannabinol ($\Delta^9$THC), cannabidiol (CBD) and cannabinol (CBN). In addition to these, the term now also encompasses both synthetic exogenous (e.g. SR141716A, a $CB_1R$ specific antagonist/inverse agonist) and endogenously produced ligands (the eCBs anandamide (AEA) and 2-arachidonyl glycerol (2-AG)) at $CB_1$- and $CB_2$Rs (for an overview of the cannabinoid receptor (CBR) ligands referred to in this review, see table 1). The development and characterisation of such pharmacological tools has enabled significant progress to be made into understanding the eCB system over the last 20 years (Onaivi *et al.*, 2002). $\Delta^9$THC, the main psychoactive component in *C. sativa,* was first isolated and synthesised in 1964 by Gaoni *et al.* (Gaoni *et al.*, 1964a). However, it was not until 1988 that the first cannabinoid specific G-protein coupled receptor (GPCR; $CB_1R$) at which it acts was characterised (Devane *et al.*, 1988; Matsuda *et al.*, 1990), lending credence to the postulated existence of an eCB system (Howlett *et al.*, 1990) (for review see Pertwee, 2005). A series of studies (Herkenham *et al.*, 1991; Herkenham *et al.*, 1990) identifying the widespread expression of this receptor in the brain further supported this discovery. These findings were rapidly followed by the isolation and structural determination of the first eCB ligand, AEA, in 1992 (Devane *et al.*, 1992), the identification and characterisation of $CB_2R$ expressed on immune cells, in 1993 (Munro *et al.*, 1993), and the identification of a second eCB ligand, 2-AG, in 1995 (Mechoulam *et al.*, 1995b; Sugiura *et al.*, 1995) (See Fig.1). Since then, significant academic and commercial effort has been invested in characterising and exploiting the eCB system

(for example reviews see Basavarajappa, 2007a; Basavarajappa, 2007b; Bisogno *et al.*, 2005; Freund *et al.*, 2003).

As previously outlined, it is now apparent that the eCB system is physiologically ubiquitous and linked to many pathophysiological states (de Fonseca, 2005), resulting in its identification as a therapeutic target, as well as the publication of numerous reviews regarding the potential benefits of cannabinoid based therapies (e.g. Agrawal *et al.*, 2009; Baker *et al.*, 2003; Glass, 2001; Goutopoulos *et al.*, 2002; Makriyannis *et al.*, 2005). This is particularly true in the field of body weight regulation and energy balance (Yates *et al.*, 2009), where significant advances have been made in the development of both anti-anorectic (e.g. in hepatitis C (Costiniuk *et al.*, 2008); in cancer (Walsh *et al.*, 2003); in human immunodeficiency virus (HIV) (Woolridge *et al.*, 2005)) and anti-obesity (Van Gaal *et al.*, 2005) cannabinoid-based therapies. However, these advances must be made with care (Grant *et al.*, 2005), and it is clear that further long-term studies are warranted if the safety profiles of such therapies are to be fully understood (Wang *et al.*, 2008). Indeed, the withdrawal of Acomplia (Rimonabant: SR141716A; see EMA, 2009 for details) from clinical use for the treatment of obesity due to serious cognitive side effects, combined with the cessation of Phase III clinical trials for a second putative anti-obesity drug, Taranabant (MK-0364; Clark, 2009), illustrates the difficulties of manipulating such a complex and ubiquitous system. Nonetheless, it is clear that the eCB system plays an important, if not yet fully understood, role in appetite, obesity and energy balance (for reviews see Cota *et al.*, 2003a; Kirkham, 2008). This review will describe the significant progress made, as well as the work still required, in order to fully understand the role played by the eCB system in feeding and appetite regulation. Importantly, by focusing on those studies that have examined pCB effects and how they

might interact with the eCB system to modulate appetite and feeding, this review provides insight into the potential use of these compounds as therapeutic tools in the treatment of eating- and weight-related disorders.

**The endocannabinoid system in the central nervous system**

Although, CBRs are present in both the peripheral and central nervous systems (CNS), the majority of cannabinoid feeding research to date has focused primarily on centrally-mediated feeding mechanisms, a focus that is consequently reflected in this review. A short section outlining the biological components that comprise the eCB system within the CNS is presented hereafter (see also figures 2 and 3).

ECBs (AEA and 2-AG) are produced on demand by postsynaptic neurons (Di Marzo *et al.*, 2004) in the CNS in response to rises in intracellular [$Ca^{2+}$] (Cadas *et al.*, 1997; Di Marzo *et al.*, 1994). Such increases typically occur following $G_q$-coupled receptor activation which stimulates production of inositol trisphosphate ($IP_3$) by phospholipase C (PLC) leading to a release of $Ca^{2+}$ from intracellular stores (for reviews see Berridge, 1995; Berridge *et al.*, 1988; Putney Jr, 1986). Alternatively, ionotropic glutamate receptor activation permits the influx of $Ca^{2+}$ to increase eCB production (Freund *et al.*, 2003).

AEA synthesis from its precursors, arachidonic acid and N-arachidonoyl phosphatidyl-ethanolamine (*N*aPE) is an on demand effect. Phosphatidylethanolamine is first catalysed by $Ca^{2+}$-dependent N-acyltransferase (Cadas *et al.*, 1997) into *N*aPE (although $Ca^{2+}$-independent processes have also been proposed; Jin *et al.*, 2007); *N*aPE is then converted to the N-acylethanolamine, AEA, (Di Marzo *et al.*, 1994) by *N*aPE phospholipase-D (Okamoto *et al.*, 2004). However, it has been suggested that at least three other pathways for AEA synthesis may also exist (for reviews, see Liu *et al.*, 2008; Okamoto *et al.*, 2009a; Okamoto *et al.*, 2009b). 2-AG is formed by the diacylglycerol (DAG) lipases α and β-catalyzed hydrolysis of DAG (Di Marzo *et al.*, 2007). After formation, eCB release into the extracellular space is thought to occur

immediately via passive diffusion or through a putative eCB transporter as no storage systems for eCBs have yet been identified and some eCB precursors are known to be membrane bound, suggesting that eCBs are produced at their release site (Di Marzo, 2008; Pope *et al*, 2010.).

Once released into the intercellular space, unlike classical neurotransmission, eCB signalling is retrograde such that eCBs released from a postsynaptic neuron diffuse across the synaptic cleft to bind with presynaptically located $CB_1$Rs to exert effects (see figure 3; Ohno-Shosaku *et al.*, 2001; Piomelli *et al.*, 2000; Wilson *et al.*, 2001) that are now thought to be intimately linked to synaptic plasticity (see Alger, 2009). The activation of presynaptic $CB_1$Rs by AEA and 2-AG induces a PLC-β-mediated suppression of $[Ca^{2+}]$ entry and a protein kinase A (PKA)-mediated upregulation of $[K^+]$ efflux, thereby driving neuronal resting membrane potential to more hyperpolarized values and so reducing the further pre-synaptic release of neurotransmitter (for review see Mackie, 2006; Südhof *et al.*, 2008). Indeed, presynaptically it has been conclusively demonstrated that $CB_1$R activation has a direct effect on membrane polarisation, for example, in cultured hippocampal neurones (Deadwyler *et al.*, 1995; Schweitzer, 2000) and in CA1 pyramidal cells (Kirby *et al.*, 2000).

ECB reuptake however remains the subject of much debate. Fatty acid amide hydrolase (FAAH; Cravatt *et al.*, 1996) and monoacylglycerol lipase (MAGL; Lambert *et al.*, 2005) are the two main enzymes involved in eCB degradation, and have been known to degrade AEA and 2-AG for some time. However, although a specific transmembrane transporter protein has yet to be identified, it seems that reuptake may be a rapid carrier-mediated transport process (e.g. Gerdeman *et al.*, 2002; Ronesi *et al.*, 2004) since eCBs within the synaptic cleft are almost immediately inactivated (Di

Marzo *et al.*, 2007). 2-AG is typically inactivated by MAGL (Di Marzo, 2008) but also to some extent by FAAH (Blankman *et al.*, 2007), whereas, AEA is thought to be degraded by FAAH alone (Vandevoorde, 2008). Four mechanisms are currently proposed to underlie eCB reuptake; a membrane-localised transmembrane carrier protein, FAAH-induced passive diffusion through the lipid bilayer, the creation of caveolae with AEA binding sites on the cell membrane which then internalise AEA and/or 2-AG, and via intracellular sequestration into the cell within a lipid shell (for a recent review see Yates *et al.*, 2009). Intracellular FAAH-mediated AEA hydrolysis creates arachidonic acid and ethanolamine (Deutsch *et al.*, 2002 thoroughly review the biochemistry of FAAH) whilst 2-AG undergoes primarily MAGL-mediated hydrolysis into arachidonic acid and glycerol (Blankman *et al.*, 2007).

While the mechanisms involved in AEA and 2-AG degradation remain poorly understood, it is clear that FAAH and MAGL blockade could be of therapeutic value. FAAH inhibition, for instance by URB597 (Piomelli *et al.*, 2006; see Vandevoorde, 2008 for a review of FAAH and MAGL inhibitors), is expected to potentiate AEA effects (and to some extent 2-AG). Such inhibition could, for instance, be expected to have effects on energy homeostasis as AEA and 2-AG administration into the hypothalamic nuclei has been conclusively demonstrated to induce hyperphagia in rats (Jamshidi *et al.*, 2001; Kirkham *et al.*, 2001a; Kirkham *et al.*, 2002). Consequently, increasing AEA concentrations in the synaptic cleft via FAAH inhibition could produce similar effects. Although MAGL inhibition (e.g. by URB602; King *et al.*, 2007) which could potentiate 2-AG effects is less well studied than FAAH inhibition, it too could be of comparable therapeutic use. It is however important to consider that both FAAH and MAGL inhibition could have secondary effects besides increasing AEA and 2-AG

levels respectively. For instance, some evidence suggests that FAAH inhibition can also decrease 2-AG levels (see Di Marzo *et al.*, 2008 for a review of these data), and MAGL inhibition, by the particularly potent and selective MAGL inhibitor JZL-184, has been found to induce behavioural effects similar to those produced by agonist-induced $CB_1R$ activation (Long *et al.*, 2009). The dynamic interplay between these systems must be taken into account when considering new therapeutic approaches if unwanted side-effects are to be minimised.

The cannabinoid receptors, $CB_1$ and $CB_2R$, are members of the superfamily of GPCRs and are characterised by seven transmembrane helices (Turu *et al.*, 2009). $CB_1R$ was cloned in 1990 (Matsuda *et al.*, 1990), 26 years after the isolation of $\Delta^9$THC. $CB_2Rs$ were first identified and characterised via polymerised chain reaction experiments in 1993 (Munro *et al.*, 1993; Onaivi *et al.*, 2006), and were initially thought to be present only in inflammatory/immune cells; Basavarajappa, 2007b; Munro *et al.*, 1993), although evidence now shows $CB_2R$ expression in the brain (Gong *et al.*, 2006; Onaivi *et al.*, 2006; Van Sickle *et al.*, 2005). Indeed, it is now known that both $CB_1$- and $CB_2Rs$ are widely expressed in the brain (Gong *et al.*, 2006; Moldrich *et al.*, 2000) although it is postulated that the majority of the functional eCB effects in the CNS are $CB_1R$ mediated (Piomelli, 2003). This receptor is one of the most abundant GPCRs in the CNS (Herkenham *et al.*, 1991) and its distribution within the mammalian brain reflects the behavioural effects of cannabinoid administration. For instance, $CB_1R$ levels are particularly high in the cortex, hippocampus, cerebellum and basal ganglia, correlating with the well-known effects of cannabinoids on cognition, memory and motor control respectively. (Herkenham *et al.*, 1991; Tsou *et al.*, 1997; for a detailed review of $CB_1R$ localisation see Mackie, 2005). Although also present in the

hypothalamus, $CB_1R$ levels are lower in this brain region than in those mentioned above (Herkenham *et al.*, 1991; Tsou *et al.*, 1997) which is somewhat surprising considering the well-known hypothalamic involvement in feeding-related processes (Elmquist *et al.*, 1999). However, a study by Breivogel *et al.* found that hypothalamic $CB_1Rs$ are more strongly coupled to G-proteins than those in numerous other brain regions including the cortex and hippocampus (Breivogel *et al.*, 1997), supporting important functional effects of cannabinoids in this brain region, and suggesting that functional gain associated with downstream intracellular signalling cascades following $CB_1R$ activation may be of crucial importance.

While $CB_1$- and $CB_2Rs$ are thought to mediate the majority of cannabinoid effects, both exo- and endo-genous cannabinoids also exert effects via other receptors. In line with this, it has been conclusively demonstrated that the non-psychotropic exogenous cannabinoids (excluding CBN and $\Delta^9$tetrahydrocannabivarin; $\Delta^9$THCV) exhibit almost no affinity for either $CB_1$ or $CB_2Rs$ (Petrosino *et al.*, 2009) and must therefore exert their known pharmacological effects via other routes. A number of alternative receptor sites are currently being investigated, including GPR55 (Brown *et al.*, 2001; Pertwee, 2007; Ryberg *et al.*, 2007), GPR119 (Overton *et al.*, 2006) and/or receptor-coupled or receptor independent ion channels (Oz, 2006) (e.g. transient receptor potential vanilloid 1; $VR_1$). The former two of these will briefly be reviewed here since both GPR55 and GPR119 activation have been found to be involved in aspects of feeding (for reviews of $CB_1$- and $CB_2R$-independent cannabinoid effects see Brown, 2007; De Petrocellis *et al.*, 2009).

GPR55, first identified by Sawzdargo in human brain and spleen and in rat brain, spleen and intestine (Sawzdargo *et al.*, 1999; for a gastrointestinal tract review

see Sanger, 2007), is currently a putative third CBR. Upon activation, GPR55 suppresses M-type $K^+$ currents and stimulates phosphatidylinositol 4,5-bisphosphate (PIP2) -induced $Ca^{2+}$ release from intracellular stores (Henstridge *et al.*, 2009; Lauckner *et al.*, 2008). While the status of GPR55 as a CBR has been a topic of fierce academic debate for some years (Petitet *et al.*, 2006), somewhat more compelling evidence has been presented recently suggesting that it is indeed a CBR (Baker *et al.*, 2006; Henstridge *et al.*, 2010; Kapur *et al.*, 2009; Lauckner *et al.*, 2005; Pertwee, 2007; Ross, 2009; Ryberg *et al.*, 2007; Staton *et al.*, 2008). Both Baker (Baker *et al.*, 2006) and Ryberg (using radioligand binding assays in human embryonic kidney (HEK293) cells; Ryberg *et al.*, 2007) have shown that CP55,940 (a non-selective CBR agonist), but not WIN55212-2 (a CBR agonist), binds with strong affinity to GPR55; Baker also showed that AEA, 2-AG and $\Delta^9$THC bind to this receptor. Moreover, Ryberg presented further evidence in the same manuscript suggesting that GPR55 was $G_{\alpha 13}$ coupled, and that the comparatively late detection of GPR55 as a possible CBR was because the affinity of CP55940 for GPR55 binding was 25 times lower than that for $CB_1R$ binding. However, in a similar study Lauckner and colleagues (Lauckner *et al.*, 2005) found that while $\Delta^9$THC could activate GPR55 in dorsal root ganglia (DRG) cells, neither WIN55212-2 nor CP55,940 bound to GPR55 in HEK293 and Chinese Hamster Ovary (CHO) cells. Furthermore the observed effects were blocked by application of SR141716A, a $CB_1R$ specific antagonist, (although not by SR144528, a $CB_2R$ specific antagonist), suggesting that these were $CB_1R$ rather than GPR55 mediated. The reasons behind the difference in CP55,940 binding between the studies by Baker, Ryberg and Lauckner are not immediately apparent, although Lauckner *et al.* suggest it may be due to a difference in GPR55 transfection between the cell types used. Despite such discrepancies, however,

evidence is mounting for GPR55's role in the eCB system, as demonstrated for instance by Kapur and colleagues, who observed AM251 ($CB_1R$ inverse agonist) and SR141716A (a $CB_1R$ specific antagonist) binding to GPR55 (Kapur *et al.*, 2009). Whilst it clearly remains an 'enigmatic' receptor (Ross, 2009), research is beginning to focus on possible GPR55 based therapies, with recent studies linking it to neuroinflammation (Pietr *et al.*, 2009), inflammatory and neuropathic pain (Staton *et al.*, 2008) and anorexia (Ishiguro *et al.*, 2010).

Finally, although the identification of GPR119 in 2003 did not initially suggest links to the eCB system (Fredriksson *et al.*, 2003), later work by Overton and Soga showing N-oleoylethanolamide (OEA), an AEA analogue, induced GPR119 activation (Overton *et al.*, 2006; Soga *et al.*, 2005) suggested otherwise. Soga *et al.* and more recently Ning *et al.* observed that GPR119 agonism mediates glucose-dependent insulin production and release to some extent, as well as increasing intercellular cyclic adenosine monophosphate (cAMP) accumulation (Ning *et al.*, 2008; Soga *et al.*, 2005). However, despite findings that GPR119 agonism by OEA suppresses feeding in rats (Rodriguez de Fonseca *et al.*, 2001), this receptor is unlikely to be involved in centrally mediated eCB feeding effects as it has been found in the gut (Overton *et al.*, 2006; Overton *et al.*, 2008). It should nevertheless still be considered when interpreting *in vivo* feeding study results and could represent a useful target for anti-obesity therapies (Hughes, 2009).

Although, as previously mentioned, this review focuses primarily on central cannabinoid mediated feeding mechanisms, peripheral effects, such as those mediated by peripheral CBRs or GPR119 add considerable complexity when interpreting *in vivo* feeding data. This is particularly true in light of findings describing peripheral $CB_1R$

expression e.g. in the vas deferens: Pertwee *et al.*, 1996; in the heart, blood vessels and bladder: Howlett, 2002; the liver and fatty tissue: Cota *et al.*, 2003b; Osei-Hyiaman *et al.*, 2005), and a potential modulatory role of these peripheral receptors on feeding behaviour (Gomez *et al.*, 2002). Equally, although it is thought that most of the functional eCB effects in the brain are $CB_1$R-mediated (Piomelli, 2003), recent work has suggested some functional expression of $CB_2$Rs in the brain (Onaivi *et al.*, 2006), particularly at synapses (Morgan *et al.*, 2009), and that these may also be involved in feeding behaviours (Onaivi *et al.*, 2008). Furthermore, CBR activation in the gut for example, in terms of gut motility (Izzo *et al.*, 2010), are also likely to have considerable effects on feeding patterns. As such, it is often difficult, when interpreting *in vivo* experimental results, to exclusively assign cannabinoid effects to either central or peripheral sites of action, or to differentiate between $CB_1$- and $CB_2$-, or indeed non-CBR-mediated effects. Moreover, ligands which produce well-characterised effects at specific CBRs may also exert effects at other receptor sites, thereby creating unexpected and potentially unwanted secondary effects. This is well illustrated by SR141716A (Acomplia/Rimonabant), a well-known selective $CB_1$R antagonist/inverse agonist which has recently been found by Kapur *et al.* to agonise GPR55, and which the authors suggest may have played a part in some of the 'off-target' effects that led to its withdrawal as an anti-obesity drug (Kapur *et al.*, 2009).

There is still much to clarify about how cannabinoids produce their endogenous effects, and how they might interact with each other *in vivo* (Ben Amar, 2006; Whalley *et al.*, 2004). Furthermore, adding to this complexity is the eCB system's interaction with other systems, in particular the opioid system which itself has been linked to appetite regulation for some time (for an early review of opiates and appetite regulation

see Morley *et al.*, 1982). Indeed, it is now apparent that both the eCB and opioid systems modulate energy balance, that both systems can modulate food intake independently and that activation or suppression of both systems together can increase or reduce food intake to a greater extent than modulation of either system alone (Gallate *et al.*, 1999; Kirkham *et al.*, 2001c; Solinas *et al.*, 2005). Whilst detailed discussion of such interactions is beyond the scope of this review, they, along with the numerous other complexities outlined above, are important factors to consider when looking at, or indeed conducting and analysing, research on the eCB system and feeding.

**Cannabinoid-mediated effects upon feeding**

The last decade has seen considerable advances in our understanding of eCB-mediated control of feeding behaviours. Numerous anecdotal reports exist, and the 'munchies' effect has become a well-accepted physiological response to *C. sativa* consumption, yet few quantitative reports from this period exist (for a complete review see Kirkham *et al.*, 2001b). While the 'classical' tetrad of cannabinoid effects was defined as decreased spontaneous motor activity, hypothermia, analgesia and catalepsy (Little *et al.*, 1988), it was only comparatively recently that $\Delta^9$THC was demonstrated to induce hyperphagia (Williams *et al.*, 1998), an effect clearly linked to $CB_1R$ rather than $CB_2R$ activation (Williams *et al.*, 2002b). Since $CB_2R$ have only recently been localised to the CNS (Morgan *et al.*, 2009), and have not been found expressed on feeding pathways, little research has considered the possible effects that $CB_2R$ stimulation may have on feeding. While a limited number of reports which consider $CB_2R$ effects on feeding do exist (e.g. Onaivi *et al.*, 2008), they have so far presented inconclusive evidence. Furthermore, possible opioid/cannabinoid and cannabinoid/serotonergic interactions have been studied, for example by Williams *et al.*, (2002b). Williams demonstrated a functional relationship between the cannabinoid and opioid systems in the control of appetite, and notably rejected any serotonergic interaction: upon administration of $\Delta^9$THC and either SR141716A, SR144528, naloxone or dexfenfluramine, Williams observed that while naloxone could block $\Delta^9$THC-mediated feeding increases, dexfenfluramine could not. Unfortunately, a detailed description of such interactions falls beyond the scope of this review, although a recent review of the effects of CB/opioid interactions on the behavioural satiety sequence can be found in Cota *et al.* (2006). Given the relatively recent success of treatment with $\Delta^9$THC-based $CB_1R$ partial agonists (e.g. dronabinol or

nabilone) and the remaining unmet clinical need in a range of disease states, further research into pCB-mediated feeding effects is on-going and is reviewed here alongside classical $CB_1R$ agonism and antagonism.

*Endocannabinoid agonism and its effects on feeding*

$\Delta^9$THC was first shown to exert hyperphagic effects in rats in 1998 (Williams *et al.*, 1998). $\Delta^9$THC (0.063-2.0mg/kg) administered orally (per ora; p.o.) to pre-satiated (a situation in which eCB tone is reduced by the highly palatable prefeed process), adult, male, Lister hooded rats, at doses >0.5mg/kg significantly increased chow intake relative to control in the first hour following drug administration. Furthermore, no significant differences in food intake between control and any $\Delta^9$THC doses were seen over the twenty four hour period following $\Delta^9$THC administration. This early study importantly demonstrated that very low concentrations of orally administered $\Delta^9$THC could substantially change an animal's motivation to consume; particularly given that a considerable percentage of the administered drug would have undergone first pass metabolism as a result of oral administration. Intriguingly, a later study by Avraham (Avraham *et al.*, 2004) also showed that extremely low $\Delta^8$THC doses (a pCB similar to $\Delta^9$THC but regarded as more stable; 0.001mg/kg; intraperitoneal; i.p.) increased feeding in mice. These studies suggest that subtle modulation of the eCB system can be achieved without the manifestation of the non-specific behavioural side effects traditionally associated with *C. sativa* administration (Howlett *et al.*, 2004). The drug administration routes used by Williams (1998; p.o.) and Avraham (i.p.) do, however, limit comparison of these results and prevent specific attribution of the effects to central or peripheral sites.

Exogenous AEA administration (0.5, 1.0, 5.0 and 10.0mg/kg; subcutaneous; s.c.) has also been observed to induce hyperphagia in pre-satiated rats (Williams *et al.*, 1999). This study demonstrated that AEA administration significantly increased food intake relative to control in the first hour of testing, in a similar fashion to $\Delta^9$THC

(Williams *et al.*, 1998), but unlike $\Delta^9$THC also induced increases in total food intake. It was proposed that the observed changes in food intake pattern represented an AEA-induced modification in the *motivation* to feed, since AEA administration caused feeding to occur far sooner than under control conditions, suggesting a cannabinoid-mediated modulation of normal feeding pathways. Indeed, close inspection of these data shows that while vehicle-treated animals consumed only small amounts of food in the first hour of testing, they consumed ten-fold more during the third hour. In slightly later work, Williams *et al.* (2002a) used two groups of twelve, male, adult, Lister-hooded rats which received $\Delta^9$THC (0.5, 1.0 or 2.0mg/kg; s.c.) or AEA (0.0, 1.0, 5.0 or 10.0mg/kg; s.c.) and video recordings of post-administration activity to investigate feeding behaviours. It became clear that administration of both $\Delta^9$THC and AEA significantly decrease the latency to feeding onset, increase the duration of intake and the number of meals, but that only AEA increased total intake. Alongside the well-reported increases in short term chow intake associated with $\Delta^9$THC, these data suggest that $\Delta^9$THC and AEA administration induce their effects by subtly different, and yet to be elucidated pathways since two distinct changes to feeding behaviours were observed.

Like $\Delta^9$THC, extremely low doses of AEA (0.001mg/kg; i.p.) have also been demonstrated to induce significant increases in food intake in female mice in experiments which used a 40% diet restriction protocol (Hao *et al.*, 2000). It is interesting that in a situation when eCB tone would already be increased due to the diet restriction paradigm used, further slight increases in eCB tone caused by extremely low doses of AEA induced changes in feeding patterns. Furthermore, it is also interesting that when Hao and colleagues administered doses of 0.7 and 4.0mg/kg AEA (also i.p.) in the same set of experiments no significant feeding effects were observed, even

though, Williams (1999) demonstrated AEA effects at 1.0 and 5.0mg/kg (s.c.). However, as the authors note, it is particularly difficult to compare Williams *et al.* (1999) and Williams *et al.* (2002a) to Hao *et al.* (2000) since the experimental paradigms are almost complete opposites. Indeed, Williams used a prefeed paradigm followed by s.c. injections in male rats and recorded food intake over a period of six hours whereas Hao used a diet restricted paradigm followed by i.p. injections in female mice and recorded daily food intake over a period of one week.

AEA feeding effects were localised to $CB_1R$ sites by Williams using a pre-satiated paradigm in 1999. Rats received AEA (0.5, 1.0, 5.0 and 10.0mg/kg; s.c.) and, in a subsequent trial, AEA (1.0mg/kg; s.c.) plus SR141716A (0.1, 0.5 and 1.0mg/kg; s.c.; Williams *et al.*, 1999). This demonstrated that AEA significantly increased food intake relative to control (in a similar fashion to $\Delta^9$THC; Williams *et al.*, 1998) and that the effect could be blocked by SR141716A, thus confirming a $CB_1R$-mediated mechanism of action for the observed feeding effects. In 2002 the hyperphagia induced by $\Delta^9$THC administration was also localised to $CB_1R$ sites (Williams *et al.*, 2002b). Williams first induced hyperphagia by administering $\Delta^9$THC (1.0mg/kg; s.c.) and in further trials attenuated this effect with the coadministration of SR141716A (0.1, 0.5 and 1.0mg/kg; s.c.) thereby demonstrating $CB_1R$ involvement in this effect. $CB_2R$-mediation was then discounted as coadministration of $\Delta^9$THC (1.0mg/kg; s.c.) and SR144528 (0, 0.05, 0.1, 0.5 and 1.0mg/kg; s.c.) failed to reduce the hyperphagia caused by $\Delta^9$THC administration.

Although Williams *et al.* (1999) localised AEA-mediated effects on feeding to $CB_1R$, an effect which one can attribute to CNS receptors, evidence which links peripheral mechanisms to these effects also exists. Gomez and colleagues (Gomez *et al.*,

2002) demonstrated that intracerebroventricular (i.c.v.) AEA administration (10.0μg/kg) and WIN55,212 (10.0μg/kg) increased food intake, but that i.c.v. SR141716A administration (0.1, 0.4, 2.0 or 10.0μg/kg) did not reduce feeding. In contrast, whilst i.p. administration of AEA (0.1, 1.0 and 10.0mg/kg) and WIN55,212 (0.4, 2.0 and 10.0mg/kg) similarly increased food intake, i.p. administration of SR141716A (0.3, 1.0 and 3.0mg/kg) reduced feeding. Furthermore, the authors demonstrated that the changes in feeding patterns they observed were unaffected by a $CB_2R$ antagonist (SR144528; 3.0mg/kg; i.p.), principally rejecting the hypothesis that these peripheral effects were in fact $CB_2R$- as opposed to $CB_1R$-mediated. SR141716A's failure to reduce feeding after i.c.v. but not i.p. administration led Gomez to suggest that $CB_1Rs$ must be present in the periphery and that these peripheral $CB_1Rs$ can affect feeding. Gomez supports this assertion by showing that intestinal AEA levels increase after food deprivation, suggesting that intestinal endocannabinergic tone plays a role in energy balance. However, the experimental paradigm used by Gomez did not account for the concentrations of SR141716A, AEA and WIN55,212 that would be present in the CNS following i.p. injection or for the considerably different dose ranges used. As such, while this evidence is suggestive of a peripheral $CB_1R$-mediated role in energy balance, further experiments are needed to confirm this.

It has been well described that upon administration of an exogenous cannabinoid agonist endocannabinergic tone will be increased and that alterations to feeding patterns can then be observed. Furthermore, it has been demonstrated that naturally occurring eCB concentrations fluctuate in various feeding states. Alongside evidence gathered using exogenous cannabinoid administration to affect feeding patterns, this elegantly demonstrates a link between how the natural eCB system modulates feeding patterns

and how artificial alterations to its state modulate the same patterns. AEA and 2-AG levels in the rat limbic forebrain, hypothalamus and cerebellum were quantified in three feeding states; fasted, feeding and satiated (Kirkham *et al.*, 2002). Four groups of between eight and ten male rats were either: 1) given *ad libitum* access to food and sacrificed during a period of low spontaneous feeding; 2) fed with a wet mash for 15 minutes and then sacrificed during feed consumption; 3) fed with a wet mash and sacrificed once they stopped eating or 4) given a 20% maintenance diet for 24hrs and sacrificed at the start of their red light cycle the following day. Brain tissue was then extracted and AEA and 2-AG levels were assessed in the limbic forebrain, hypothalamus and cerebellum. AEA and 2-AG levels were significantly elevated by food deprivation in the limbic forebrain while 2-AG concentration was significantly reduced in the hypothalamus during the feeding state but significantly increased during the deprived state. Since 2-AG levels were increased in the limbic forebrain and hypothalamus, and AEA levels were significantly increased in the limbic forebrain, it appears that elevated eCB levels in important reward-related brain areas during food deprivation play a role in motivating animals towards food. The reduction of 2-AG levels in the hypothalamus during feeding suggests that 2-AG is actively suppressed during feeding to facilitate satiation. Such data suggest subtly differing roles for AEA and 2-AG in feeding cycles.

Further experiments published in the same manuscript revealed that 2-AG (i.c.v. into the nucleus accumbens at 0.5 and 2.0μg) could significantly increase food intake over a one hour period while coadministration of SR141716A (0.5mg/kg; s.c. plus 0.5μg 2-AG; i.c.v.) could almost fully attenuate this effect, thereby demonstrating $CB_1R$ mediation. The increases in food intake seen in Kirkham *et al.* (2002) are comparable to

the similar studies performed by the same group following $\Delta^9$THC administration (Williams *et al.*, 2002b; Williams *et al.*, 1998), however are considerably different from a feeding study which administered AEA (Williams *et al.*, 1999). Indeed, AEA (1.0mg/kg; s.c.) effects were observed over four hours and during the first hour significantly increased intake by a factor of approximately two, as well as significantly elevating food intake over the entire four hour period. In contrast, 2-AG (0.5 and 1.0μg/kg; i.c.v.) significantly increased food intake by factors of nine and seven, respectively, over a one hour period. However, it is important to note that in this instance 2-AG administration was i.c.v. and AEA administration was s.c., and it is possible that AEA-mediated feeding increases would have been larger had administration been i.c.v. Given the strong anatomical connections between the nucleus accumbens shell and the hypothalamus (Stratford *et al.*, 1999) and the importance of the hypothalamus in the integration of feeding pathways (Maccarrone *et al.*, 2010) these data represent a demonstration that modulation of this pathway can have profound feeding effects.

As considerable differences in eCB levels in the limbic forebrain were seen during various feeding states in (Kirkham *et al.*, 2002) and because the limbic forebrain is traditionally associated with reward processing, possible interactions between the eCB and opioid systems were then considered by Williams and Kirkham (Williams *et al.*, 2002b). It was demonstrated that $\Delta^9$THC-induced hyperphagia could be attenuated by the opioid antagonist naloxone. This proves a link between opioid food reward processing and the eCB system. These experiments are also exciting in terms of putative anti-obesity treatments. Indeed, it has been reported that in obese humans, eCB levels are elevated by up to 52% (Engeli *et al.*, 2005); as such experiments which artificially

elevate eCB activity using food-restriction paradigms are particularly valuable since they more accurately model the human obese condition. Moreover, since in both the food deprived and obese states eCB levels are increased, this further supports the theory that eCB dysfunction plays an important part in human obesity. Further studies which directly compared AEA and 2-AG-induced feeding effects (both centrally and peripherally administered) would be extremely useful. It would be equally compelling for further work to consider fully the putative effects of peripheral cannabinoid receptors on feeding behaviours, for example in terms of gut motility.

Given the previously discussed alterations to eCB concentrations in the limbic forebrain, an area traditionally associated with reward processing and well documented opioid/cannabinoid interactions, it is clear that the changes to behavioural patterns observed during cannabinoid administration are due to changes in reward levels. Therefore, cannabinoid agonist effects on the consumption of different types of rewarding ingesta, such as sweet versus fatty foods, was addressed in a study by Koch (2001). Here, $\Delta^9$THC (0.0, 0.5, 1.0 and 2.5mg/kg; i.p.) was administered to rats receiving either control (chow), high fat (HF) or high fat plus sugar (HFS) diets and food intake was recorded over 24 hours post-injection. After the first hour, 0.5 and 1.0mg/kg $\Delta^9$THC significantly increased food intake versus control within the HF diet group only, while doses of 2.5mg/kg had no significant effects on intake in any group. However, overall food intake during the first hour was significantly greater in the HF group than in either the control chow or HFS groups. During the second hour of testing, the 0.5 and 1.0mg/kg $\Delta^9$THC doses significantly increased intake versus control in all three groups with 1.0mg/kg causing the largest increases. This dose also induced a significantly greater intake increase in the HF (but not in the HFS) than in the control

chow group, although overall food intake across all doses was significantly greater in both the HF and HFS groups, relative to the control chow group. Finally in the fourth hour, unlike previous feeding studies employing $\Delta^9$THC which showed effects lasting fewer than four hours, $\Delta^9$THC doses of 1.0mg/kg still induced significant increases in consumption compared to vehicle in the HF and HFS diet groups.

The increases in intake seen at lower dose levels by Koch appear comparable (though reduced) to those previously reported (Williams *et al.*, 1998) and support a cannabinoid-mediated stimulation of intake (Williams *et al.*, 1999; Williams *et al.*, 1998), although the non-significant effects of 2.5mg/kg $\Delta^9$THC remain unexplained, particularly as no changes to motor coordination or the appearance of tetrad effects were reported for this dose. Therefore, it is clear from these results that eCB system stimulation induces greater increases in the intake of highly fatty food-types (HF) than other, less calorific or more palatable, diets (HFS). Previous work by Arnone *et al.* (1997) and Simiand *et al.* (1998) (this work is fully discussed in 'Endocannabinoid antagonists and their effects on feeding') would suggest that the HFS intake should also have been increased by $\Delta^9$THC administration. Indeed, $\Delta^9$THC administration induced similar intake increases in the HFS and HF diet groups but only after the fourth and twenty-fourth hours of testing. These data could suggest that the eCB system is involved more strongly in increasing the motivation to consume high fat foods and that this effect manifests more quickly than eCB increases due to other 'bland' food types. From the perspective of evolution, an increased motivation to consume high fat foods is obvious and as such it is reasonable to suggest from this data that the eCB system is intimately involved in this process.

In a later study specifically designed to look at cannabinoid modulation of palatability, Higgs *et al.* (2003) tested the effects of various cannabinoid receptor ligands on consumption of a 10% sucrose solution in rats. In this test $\Delta^9$THC or AEA (0.5, 1.0 or 3.0mg/kg in both cases; i.p.) but not 2-AG (0.2, 0.5 and 2.0mg/kg; i.p.) significantly increased sucrose solution consumption. By analysing lick patterns using the mathematical model of ingestive control proposed by Davis *et al.* (1977), this effect was ascribed to a cannabinoid-induced increase in perceived palatability. The lack of significant 2-AG-induced effect is surprising, since both 2-AG and AEA are partial agonists at $CB_1R$ sites (although it has been suggested that 2-AG is the main ligand associated with $CB_2R$ sites; Sugiura *et al.*, 1999; Sugiura *et al.*, 2000), and because of the significant effects observed by Kirkham *et al.,* (2002). Higgs suggests that different speeds at which the exogenous and endogenous ligands act, coupled with the probable lack of 2-AG brain penetration (due to the route of administration), can explain the reduced effects of AEA compared to $\Delta^9$THC and could, to some extent, shed light on the limited effects of 2-AG. Administration of SR141716A alone was also tested (0.5, 1.0 and 3.0mg/kg; i.p.) by Higgs, and found to elicit a dose-dependent decrease in consumption, thus linking the observed effects to modulation of endogenous $CB_1R$ activation. Yet, it could be argued that the dose-dependent decreases in consumption induced by SR141716A seen here were caused by a reduction in eCB tone of which 2-AG plays a part, and as such it remains impossible to discount any effects of 2-AG acting at $CB_2R$ sites on this process. This finding demonstrates that the eCB system must have an active effect upon appetite and feeding since $CB_1R$ blockade alone, without co-administration of a cannabinoid agonist (c.f. Gallate *et al.*, 1999; Williams *et al.*, 1999), significantly reduces consumption. Considering the works of Koch (2001),

Gallate *et al.* (1999) and Williams *et al.* (1999) it can be suggested that eCB system stimulation upregulates the palatability of high calorie, low palatability foods and conversely, reducing endocannabinergic tone diminishes perceived palatability. This could explain why in Koch's work the HF diet intake was increased above HFS diet as it was both palatable and calorie-rich.

In a very recent study Yoshida and colleagues (2010) administered AEA and 2-AG to both wild type and $CB_1R$ knock-out mice. They observed that AEA and 2-AG preferentially increased taste responses to sweet rather than salty, sour, bitter or umami (a Japanese term for a meaty or savoury taste) flavours in the chorda tympani nerve, fungiform taste buds and during a short-term lick response test. Furthermore, they did not observe this effect in their knock-out mice, hence linking this effect to $CB_1R$. Given their i.p. route of administration, the authors have suggested a peripheral effect, alongside the well described central effect, of eCBs on taste. In comparison with the work of Higgs *et al* (2003) who found 2-AG to have little effect on palatability in a progressive lick-based paradigm, and considering that both studies used i.p. administration and observed peripheral taste responses, it is not immediately apparent why different eCBs are identified as the principal modulators of taste.

In earlier taste reactivity studies, Gallate *et al.* (1999) presented rats with various concentrations of 'beer' (<0.5%, 2.7% or 4.0% ethanol solutions) or an 8.6% sugar solution, and their motivation to consume these substances was measured using a lick-based progressive ratio paradigm. In order to assess $CB_1R$ involvement, animals were treated with either vehicle, the $CB_1R$ agonist CP55,940 (10.0, 30.0 or 50.0μg/kg; i.p.), CP55,940 (30.0μg/kg; i.p.) plus SR141716A (1.5mg/kg; i.p.), or naloxone (2.5mg/kg; i.p.) plus CP55,940 (30.0μg/kg; i.p.). This drug regime therefore also examined

potential interactions between the cannabinoid and opioid systems. In agreement with previous research, CP55,940-treated animals exhibited a significant trend whereby increasing dose led to increases in both beer and sucrose solution consumption. SR141716A-induced $CB_1R$ blockade during CP55,940 treatment attenuated this effect, reducing beer solution intake to levels that did not differ from vehicle treatments. Furthermore, CP55,940 plus naloxone administration significantly reduced beer intake to approximately 50% of vehicle intake, confirming a strong interaction between $CB_1$ and opioid receptors in the control of alcohol consumption. This study demonstrated that $CB_1R$ activation could induce significant increases in palatable solution intake when compared to vehicle-treatments, and that this effect was attenuated by $CB_1R$ antagonists; further supporting the theory of $CB_1R$ involvement in perceived palatability.

In 2005 and 2007 Jarrett *et al.* (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007) demonstrated that the eCB system mediated the perceived palatability of sweet and bitter tastes. In trials using adult, male Sprague-Dawley rats, $\Delta^9$THC (0.5mg/kg; i.p.), $\Delta^9$THC (0.5mg/kg; i.p.) plus SR141716A (2.5mg/kg; i.p.) or AM251 (1.0mg/kg; i.p.), or AM251 (1.0mg/kg; i.p.) alone were administered via intraoral cannulae and the orofacial reactions to intraoral administration of either sucrose (2, 10 and 32%), or quinine (0.01 or 0.05%) solutions were recorded. $\Delta^9$THC increased the perceived palatability of the sucrose solution, an effect attenuated in animals treated with $\Delta^9$THC plus SR141716A (Jarrett *et al.*, 2005). In the later study, $\Delta^9$THC reduced aversiveness to 0.05% quinine solution, an effect that was blocked by coadministration of AM251 (Jarrett *et al.*, 2007). The authors suggest that their results indicate that increases in $CB_1R$ responsiveness could be involved in a general upregulation of the ingesta

palatability. In further experiments during the same two studies, Jarrett and colleagues also demonstrated that SR141716A administration alone does not reduce perceived palatability (Jarrett *et al.*, 2005) but AM251 administration enhances aversiveness (Jarrett *et al.*, 2007). This may suggest that the increased palatability of sweet food can activate the eCB system and upregulate eCB tone. However, in the case of bitter tasting food, when eCB tone would not be increased by the low palatability of the food, $CB_1R$ antagonism can then reduce the perceived palatability of the food further.

Further supporting this palatability hypothesis, it has recently been demonstrated that, under a reinforced learning paradigm which included food type (normal lab chow, chocolate pellets or fat-enriched pellets) selection as an experimental variable, mice will select the sugar rich, highly-palatable food type or fatty foods over standard food after $\Delta^9$THC (1.0mg/kg; i.p.) administration (Barbano *et al.*, 2009). Since, the chocolate-enhanced pellets used in this study had the same calorific content as the standard food pellets also employed while the fat enriched pellets contained approximately 66% more calories per gram but no added chocolate, it can be observed that $\Delta^9$THC administration increased the palatability of fatty foods to a level comparable to that of sweet foods.

The conclusion that the eCB system exerts a level of control over palatability reward is well supported. It has been shown that increased eCB tone increases the palatability of sweet liquids (Gallate *et al.*, 1999; Higgs *et al.*, 2003), bland fatty foods (Koch, 2001) or even aversive, bitter liquids (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007). Indeed, eCB levels change naturally reflecting the current feeding state, and it is this basal tone that can be altered with the application of $CB_1R$ ligands. Furthermore, the meal pattern changes induced by stimulation of the eCB system are also well understood. $\Delta^9$THC administration has been demonstrated to reduce the latency to

feeding onset in periods of reduced endocannabinergic tone and has also been shown to increase meal sizes. If we consider appetitive behaviours as those that regulate the latencies to meal onset and consummatory behaviours as those that regulate the size of any given meal, what becomes clear from these data is that modulation of the eCB system can affect consummatory *and/or* appetitive behaviours and that these different behavioural changes can be manipulated individually. For example, in the work of Williams *et al.* (2002a), a distinct separation was seen between the AEA and $\Delta^9$THC groups in terms of feeding behaviour. In this instance, AEA administration produced effects comparable to $\Delta^9$THC: AEA significantly reduced the latency to feeding onset, whilst also increasing the number of eating bouts. AEA administration also increased meal duration and the duration of the longest bout versus control conditions. However, unlike $\Delta^9$THC, AEA induced significant increases in total intake over the test period. Given these data it can be clearly seen that the changes to feeding patterns (either in gross terms or in terms of palatability levels) are due to changes to reward processing. Indeed, the decreased latency to feeding onset and the increased size and length of meals thereafter coupled with increased lick responses can all be linked to increased reward.

*Endocannabinoid antagonism and its effect on feeding*

Alongside the well documented hyperphagic effects of $\Delta^9$THC administration there is comprehensive complimentary evidence which details the effects of reducing eCB tone on feeding behaviours. Indeed the use of $CB_1R$ antagonist-based studies represents an elegant way to study the effects of basal eCB tone on physiological feeding mechanisms. This is particularly true as many CBR antagonists are highly selective. In 1997 Arnone (Arnone *et al.*, 1997) and colleagues performed a comprehensive set of experiments which revealed the changes to feeding behaviours induced by $CB_1R$ blockade by the administration of SR141716A (0.0, 0.1, 0.3, 1.0 or 3.0mg/kg: i.p.). In experiments which studied either intake of sucrose pellets, intake of a 5% sucrose solution or a 10% ethanol solution, Arnone demonstrated that eCB tone plays an important role in the perceived appetitive values of both alcohol and sugar. Firstly, Arnone and colleagues measured the weight of food consumed in 30min open field tests where food-restricted male, Wistar rats (a situation in which one would expect eCB tone to be increased by the food restriction (Kirkham *et al.*, 2002)) had access to either sucrose pellets and standard lab chow or standard lab chow alone after SR141716A administration. The quantity of sucrose pellets consumed by rats was reduced from 3.1±0.4g under control conditions to 1.0±0.4g after the 3.0mg/kg SR141716A dose in a dose-dependent fashion; furthermore, the two highest doses of SR141716A induced significant reductions when compared to control intakes. Standard chow intake was unaffected. However, when Arnone repeatedly administered 3.0mg/kg SR141716A in mice with a predisposition to alcohol consumption over four, six hour test periods in one day, SR141716A induced a suppression of ethanol intake. Secondly, rats (male, Sprague-Dawley) were given access to a 5% sucrose solution for a period of four hours

without access to food and water. As a control experiment another group of rats was given access to water for the same test period. Sucrose solution intake was significantly reduced by the two highest doses of SR141716A and a significant dose-dependent reduction in sucrose solution intake was apparent. Water intake was unaffected by SR141716A administration at any dose. Thirdly, Arnone tested the effect SR141716A had on ethanol consumption in male C57BL6 mice; once again administration of either 1.0 or 3.0mg/kg SR141716A induced significant reductions in consumption versus control-treatment and a significant dose-dependent effect was apparent for the entire dose range. Water-only tests were used as a control, as per the 5% sucrose solution test, and no effect of SR141716A was apparent. These three experiments demonstrate that the eCB system plays an active role in the control of the appetitive value of sweet and alcoholic ingesta. Indeed, such experiments prove that the eCB system must have a constantly active basal tone as antagonists applied to an inactive system would induce no behavioural changes alone. Furthermore, given the selectivity of SR141716A we can be confident that this effect is localised to $CB_1R$ sites.

Reductions in eCB tone induced by the administration of SR141716A (0.0, 1.0 and 3.0mg/kg; p.o.) were extended to marmosets in 1998 by Simiand (Simiand *et al.*, 1998). Marmosets were habituated to a high-sugar mash (HSM; 33% sugar, 67% milk and cereal) and after habituation administered SR141716A twice a week; their food (either HSM or standard diet) intake was then observed for a period of six hours. Administration of the 3.0mg/kg dose induced significant reductions in HSM intake versus control-treatments after one, three and six hours while the 1.0mg/kg dose only reduced HSM intake versus control-treatments during hours one and three. Notably standard diet intake was unaffected by SR141716A administration with the exception of

a significant increase in intake seen during hour six. These data demonstrate that SR141716A can preferentially reduce intake of sweet, highly palatable food, versus bland food. This suggests that eCB tone is responsible for increasing the appetitive values of high-sugar foods under normal physiological conditions. Indeed, as previously discussed, such work has been supported by later experiments which showed SR141716A administration could reduce the reward associated with sucrose solutions (Higgs *et al.,* 2003). Since Arnone *et al*. (1997) and Simiand *et al.* (1998), together, used two strains of rats, one strain of mouse and one type of primate and observed similar results, it can be suggested that the effects of the eCB system are similar across species. However, the work of Arnone and Simiand did not consider a high fat diet alternative to the high-sugar foods they used, and they only antagonised the eCB system over a shorter period of time. It is worthwhile comparing the works of Arnone and Simiand to that of Williams and colleagues. Indeed, while Williams observed $CB_1R$ stimulation to increase feeding, and that this effect could be blocked by coadministration of SR141716A, Arnone and Simiand demonstrated a basal endocannabinergic tone which when blocked with SR141716A alone could reduce feeding. Furthermore, as discussed, the eCB system has been demonstrated to alter its activity level dependent on feeding state (e.g. deprived of food versus eating; Kirkham *et al.,* 2002). Taken together, these studies demonstrate that eCB tone is a powerful effector of perceived hunger and that feeding reduces endocannabinergic tone in the same way as blocking $CB_1R$ sites in the CNS.

$\Delta^9$THC-induced effects on high fat versus high sugar foods were well described by Koch in 2001. However, only recently were antagonist studies used to facilitate the understanding of the role of $CB_1R$ alone in the selection and consumption of highly

fatty or sweet foods. In 2007, a study by Thornton-Jones *et al.* (2007) examined the effects of SR141716A-induced $CB_1R$ blockade on consumption of sweet (10% sucrose) or fatty (10% intralipid) solutions and analysed the lick-based response in a way similar to Higgs *et al*. (2003). Appealingly, this paradigm also compared the hypophagic SR141716A effects with those induced by behavioural manipulations designed to reduce either motivation to feed (pre-feeding) or food palatability (addition of quinine to the intralipid solution and reduction of sucrose concentration to 5%) which, combined with the lick-based microstructure analysis, allowed for more detailed interpretation of the results. Supporting previous findings, this study demonstrated that SR141716A treatment reduced both sucrose and intralipid solution intake, and microstructure analysis revealed that these reductions were due to decreased perceived palatability of ingesta. However, whilst these significant reductions were induced by both 1.0mg/kg and 3.0mg/kg SR141716A for the intralipid solution, only 3.0mg/kg SR141716A significantly reduced sucrose solution consumption. Furthermore, reduced lick duration of intralipid solution observed after drug administration led the authors to suggest that the SR141716A-induced reduction in consumption of this solution was a result of a decrease in motivation to feed, as well as an effect on perceived palatability. In contrast, reductions in sucrose solution intake were less pronounced than those seen for the intralipid solution, suggesting that eCBS may be more strongly implicated in consumption of highly calorific than sweet, palatable food-types. Taken alone, this work suggests that the eCB system can play its role in energy homeostasis via modulation of the motivation to consume fatty foods specifically; this long term effect may be due to changes in meal patterning over the shorter term, particularly with respect to the control of fat intake. Indeed, the work of Thornton-Jones further supports the findings of

Arnone *et al.* (1997), Simiand *et al*. (1998) and Koch (2001), as previously discussed, and strengthens the link between $CB_1R$ activation and the control of feeding.

Using a different experimental approach, a recent study by Mathes *et al.* (2008) also addressed these issues, and provided further support for eCB system involvement in the specific consumption of highly fatty and/or sweet foods. A 'dessert protocol' was employed, whereby rats were given *ad libitum* access to a wet mash diet made from standard laboratory chow in addition to simultaneous but time limited access to a 'dessert' diet high in fat and sugar. Vitally, this methodology gave rats a choice between two different diets, allowing within-group analysis of diet selection. eCB system involvement in the consumption of these two different food-types was assessed by administration of either of the $CB_1R$ antagonists, SR141716A (1.0mg/kg; i.p.) or AM251 (0.3, 1.0 or 3.0mg/kg; i.p.) 30min prior to dessert access. Both compounds significantly reduced caloric intake over a 24 hour period, but this effect was due to a significant decrease in the consumption of the high fat and sugar diet only, with no effect on the consumption of the standard chow. When considered alongside Arnone *et al.* (1997), Koch (2001), Simiand *et al.* (1998) and Thornton-Jones *et al.* (2007), these findings suggest that $CB_1R$-mediation of intake not only modulates intake of fatty food more that sugary food, but does so in a preferential fashion. Unfortunately, although Mathes *et al.*, in the earlier stages of this study, compared the consumption of the high fat and sugar diet with another diet high in sugar only, the effects of $CB_1R$ blockade on this sugar-only diet were not examined.

While the use of the previously discussed antagonists has been shown to reduce feeding in a number of models, there is considerable on-going research looking for novel compounds which perform similar actions. Such research is performed to further

our understanding of the eCB system and its ligands, whilst also searching for new anti-obesity drugs (for examples please see PSNCBAM-1, Horswill *et al.*, 2007; AM4113, Sink *et al.*, 2007; O-2050, Gardner *et al.*, 2006; MK55-96, Yan *et al.*, 2010; and MK-0364, Fong *et al.*, 2007). While, O-2050 was found to induce significant reductions in motor coordination, which may have been the cause for its effects on feeding behaviours, PSNCBAM-1, AM4113 and MK-5596 have been demonstrated to reduce short term intake and reduce body weight in animal models without apparent side effects. Further work using these novel agents would benefit from the inclusion of meal patterning analysis as this would allow the study of appetitive and/or consummatory behaviours, as well as effects on perceived palatability; such an analysis may shed more light on eCB mediation of feeding behaviours.

*Non-$\Delta^9$THC phytocannabinoid effects on feeding*

Very recently it has been suggested that non-$\Delta^9$THC pCBs can also modulate rodent feeding patterns (Farrimond *et al.*, 2010a; Farrimond *et al.*, 2010b) and induce different changes than $\Delta^9$THC, without any apparent non-specific behavioural effects. Before 2009 there had been a limited number of studies which demonstrated non-$\Delta^9$THC pCB-mediated feeding effects. Unfortunately these studies were either unrepeated or contradictory. Sofia and Knobloch (1976) reported that the $CB_1R$ agonist CBN reduced intake in rats, an effect yet to be repeated, and that CBD also reduced feeding in rats. However, Wiley *et al.*, (2005) reported that CBD administration had no effect on food intake in mice, a result repeated by Riedel (2009) who observed only a small, non-significant reduction in food intake and weight gain, also in mice. It should be noted that the dose ranges used by Sofia, Knobloch and Wiley are considerably higher than those used by Farrimond and colleagues and, given the well described bi-phasic dose effects of $CB_1R$ activation (Glick *et al.*, 1972) on feeding, this may explain the differences in observed effect patterns. Work in our laboratory has demonstrated that a range of pCBs may have significant effects on feeding patterns. Thirty-six pre-satiated, male, Lister-hooded rats received a purified, *C. sativa*-derived $\Delta^9$THC (hereafter purified $\Delta^9$THC), a synthetic $\Delta^9$THC and a high-$\Delta^9$THC standardised botanical drug substance (BDS), similar in composition to that used in Sativex and containing a typical array of non-$\Delta^9$THC pCBs (high-$\Delta^9$THC BDS; all treatments were dose matched for $\Delta^9$THC content; 0.00, 0.34, 0.67, 1.34 and 2.68mg/kg; p.o.; Farrimond *et al.*, 2010a). Synthetic and purified $\Delta^9$THC administration induced classical $\Delta^9$THC hyperphagia in this study: the latency to the first meal was reduced and both the first hour intake and first meal duration were increased versus vehicle treatments. Intriguingly, the effects of

the high-$\Delta^9$THC BDS administered showed significantly reduced hyperphagia in comparison. These results suggested that the combination of pCBs (and, potentially, non-cannabinoid components) in the high-$\Delta^9$THC BDS attenuated the hyperphagic effects of $\Delta^9$THC. Given the constituents of the high-$\Delta^9$THC BDS, ($\Delta^9$THC; 67.0%, Cannabigerol (CBG); 1.7%, Cannabichromene (CBC); 1.6%, CBN; 1.5%, $\Delta^9$THCV; 0.9%, $\Delta^9$tetrahydrocannbinolic acid ($\Delta^9$THCA); 0.3%, CBD; 0.3% and Cannabitriol (CBO); 0.2%) it is possible that the pCBs present could have affected feeding synergistically, or that at least one pCB modulated the $\Delta^9$THC-induced hyperphagia. Indeed, the high-$\Delta^9$THC BDS used in Farrimond *et al.*, (2010a) contained known $CB_1R$ antagonists ($\Delta^9$THCV, Dennis *et al.*, 2008; Ma *et al.*, 2008; Thomas *et al.*, 2005) in addition to modulators of $Ca^{2+}$ homeostasis (CBD; Ryan *et al.*, 2009) and CBN (Rao *et al.*, 2006); which could modulate eCB levels. However, these results do not preclude the possibility that non-cannabinoid material also present in the high-$\Delta^9$THC BDS also contributed to the observed behavioural effects.

Consistent with these results we have also demonstrated that non-$\Delta^9$THC pCBs can affect feeding when co-administered with sub-effective concentrations of $\Delta^9$THC (Farrimond *et al.*, 2010b; please also see Riedel *et al.*, 2009 for non-$\Delta^9$THC pCB-induced hypophagia fully discussed below). Ten, pre-satiated, adult, male, Lister-hooded rats received a low-$\Delta^9$THC standardised BDS (low-$\Delta^9$THC BDS; 0.0, 0.5, 1.0, 2.0 and 4.0mg/kg; p.o.) containing only 6.9% $\Delta^9$THC, alongside 14.2% other pCBs. Conversely, at these concentrations, rather than having no effect on feeding (as it might be expected, given the use of a prefeed paradigm with an extremely low dose of $\Delta^9$THC and pCBs that have previously been shown to reduce $\Delta^9$THC-mediated hyperphagia), the low-$\Delta^9$THC BDS significantly increased chow intake during the first hour, by

significantly reducing the latency to the first contact with food for the highest doses versus vehicle-treatments. These reductions in meal one latency mirror those previously demonstrated for considerably higher concentrations of pure $\Delta^9$THC (Farrimond *et al.*, 2010a; Williams *et al.*, 1998). Furthermore, while the latency to the first meal was significantly reduced, the size and duration of this meal was unaffected. Therefore, we suggested that the ratio of non-$\Delta^9$THC pCBs present in the low-$\Delta^9$THC extract significantly increased appetitive (but not consummatory behaviours), since the concentrations of $\Delta^9$THC present were between two- and ten-fold lower than those previously demonstrated to induce hyperphagia (Farrimond *et al.*, 2010b).

When these two studies are considered together (Farrimond *et al* 2010a and 2010b), a number of suggestions can be made: firstly, since the observed effects of the non-$\Delta^9$THC components of the extracts administered reduced hyperphagia when presented in one ratio and increased hyperphagia when presented in a different ratio, it is highly unlikely that the plant matter also present was responsible for these effects, given that its content did not change between the two tests. Secondly, it is possible that the pCBs present in both studies did not act in isolation such that more than one compound induced the observed effects. Thirdly, these effects could involve non-CBR dependent pathways since several of the non-$\Delta^9$THC pCBs present are known to act via mechanisms only partially connected to CBR (see Izzo *et al.*, (2009) for review). Finally, these results give further credence to the already well supported therapeutic potential of non-$\Delta^9$THC pCBs for the treatment of energy balance disorders. Indeed, non-$\Delta^9$THC pCB-based treatment may prove more tolerable than $\Delta^9$THC treatment alone (e.g. Nabilone) due to the absence of psychotropic side effects associated with non-$\Delta^9$THC pCBs.

Whilst a definitive mechanism underlying the findings reported by Farrimond *et al.*, (2010a and b) remains unknown, the naturally occurring pCB and $CB_1R$ neutral antagonist $\Delta^9$THCV, which was also present in the extracts employed in Farrimond *et al.*, has been considered alone and shown to exert hypophagic actions (Riedel *et al.*, 2009). Indeed, given the difficulties associated with the use of the highly specific and potent $CB_1R$ antagonist/inverse agonist SR141716A in the clinic, the use of $\Delta^9$THCV, as a neutral antagonist, to reduce food intake may be of therapeutic value. Riedel *et al.* (2009) demonstrated that purified $\Delta^9$THCV (3.0, 10.0 and 30.0mg/kg; i.p.) significantly reduced chow intake over a period 12 hours and induced weight loss in male C57BL6 mice. Subsequent treatment with extremely low $\Delta^9$THCV BDS doses (which contained between 0.1 and 0.3mg/kg $\Delta^9$THC) failed to induce changes to feeding patters; however the $\Delta^9$THC quantities presented were within the range previously observed to affect meal patterning in rats (Farrimond *et al.*, 2010). Unfortunately, meal microstructure was not analysed in this study. In a further experiment, Riedel hypothesised that if CBD (10.0mg/kg; i.p.) were to be co-administered with these very low $\Delta^9$THCV BDS doses, the effects of the present $\Delta^9$THC would be antagonised, and the hypophagic properties previously induced by purified $\Delta^9$THCV would therefore emerge. However, the evidence for *in vivo* antagonism of $\Delta^9$THC by CBD has so far only been demonstrated in a memory task (Fadda *et al.*, 2004), and Riedel observed no significant effects on feeding or weight change following CBD co-administration. The authors suggest that this lack of effect may also be attributed to other pCBs, present in his extract, or simply because the concentrations of $\Delta^9$THCV administered were too low. However, Riedel recorded all feeding data during the light phase of the rats' day during which time little feeding typically occurs. Furthermore, it is also possible that the experimental paradigm

used was not optimised for the observation of hypophagia. For instance, introducing food-deprivation prior to dosing could accentuate possible differences between control and conditions measurements, thereby rendering the experimental paradigm more sensitive to $\Delta^9$THCV-mediated reductions in feeding. While, Riedel has confirmed that purified $\Delta^9$THCV can reduce food intake in mice, further experiments which use more sensitive paradigms and analyse meal microstructure after $\Delta^9$THCV BDS administration may prove useful in fully understanding Riedel's data.

The work discussed throughout this manuscript demonstrates that some pCBs can alter appetitive and consummatory behaviours separately (e.g. Higgs *et al.*, 2003). As such if it were possible to use at least one non-$\Delta^9$THC pCB to modulate consummatory behaviours (to increase or decrease the quantity consumed during a natural eating event) rather than inducing immediate short term post-administration hunger (the 'munchies', a colloquial term meaning increased hunger normally due to smoking cannabis recreationally) it may be possible to produce a *C. sativa*-based medicine which could control absolute intake quantity. Even though the mechanisms by which the eCB system modulates palatability and therefore diet selection remain unclear, direct effects on diet selection caused by cannabinoid administration have also been demonstrated. Given the presented data which suggests that increased eCB tone leads to increased selection of fatty foods over sweet foods, it is a possibility that non-psychotropic pCBs could also be used to modulate diet selection i.e. to reduce fatty food palatability in obesity. Indeed, putative anti-obesity treatments using pCBs are of great interest. Equally, while some pCBs have been shown to induce significant reductions in food intake and in some cases reduce body weight (e.g. $\Delta^9$THCV), little research is

currently considering possible mechanisms by which intake and body weight may be increased, which would be valuable in the treatment of anorectic disorders.

## Conclusions

A considerable quantity of characterisation work has been performed on $\Delta^9$THC-mediated feeding effects, but the specific roles of other pCBs remain less well understood. Work in our laboratory and others suggests that non-$\Delta^9$THC pCBs can have profound effects on feeding behaviours (Farrimond *et al.*, 2010a; Farrimond *et al.*, 2010b; Riedel *et al.*, 2009; Sofia *et al.*, 1976; Wiley *et al.*, 2005). However, these are not yet well characterised. As such considerable further work is required to assess the effects of both purified single pCBs as well as combinations of numerous pCBs. With the recent UK approval of the $\Delta^9$THC CBD combination in Sativex, it can be clearly seen that the use of pCBs in the clinic is gaining public acceptance that regulatory bodies are willing to accept plant-derived medicines alongside their synthetic counterparts. Indeed, non-$\Delta^9$THC pCBs may represent an untapped clinical potential for a number of feeding disorders. Such a need is particularly important given the non-specific behavioural side effects associated with the use of $\Delta^9$THC and its analogues in the clinic. Given the encouraging results demonstrating non-$\Delta^9$THC pCB-mediated modulation of feeding, it is tempting to suggest that these compounds may provide at least the basis for novel therapies for the treatment of feeding disorders.

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**Figure 1;** the development of cannabinoid pharmacology 1964 – 2010.

**Figure 2;** eCB signalling pathways. Upon invasion of an action potential into the synaptic region of the presynapse, the release of neurotransmitter (in this case glutamate or acetylcholine) and the binding of neurotransmitter to postsynaptic receptor sites: the eCBs AEA and 2-AG are produced on demand from arachidonic acid by *N*aPE and DAG respectively and released into the synaptic cleft from the postsynapse (red). Upon neurotransmitter binding to both metabotropic Glutamate and Acetylcholine receptors G subunits disassociate and stimulate an increase in PLC-β levels. This leads to an upregulation of intracellular $IP_3$ which gates calcium channels on the endoplasmic reticulum thus increasing intracellular $Ca^{2+}$. Furthermore, the release of $G_{\alpha i/o}$ subunits upregulates the production of cAMP and therefore allows further $Ca^{2+}$ entry into the cell via VGCCs. Equally upon activation of iGluR channels calcium is allowed into the cell. Once released into the extracellular space the endocannabinoids bind to $CB_1R$ sites on the presynapse (green) causing the presynaptic cell to move closer to its resting membrane potential principally by closing VGCCs and opening VGPCs thus reducing the available intracellular $Ca^{2+}$ required to facilitate further neurotransmitter release.

**Figure 3;** simplified diagram illustrating retrograde signalling by eCBs in the CNS. Following vesicular neurotransmitter release from the presynaptic cell (green), neurotransmitters bind to receptor proteins (black) on the postsynaptic cell (red) stimulating eCB production and release from the postsynaptic cell. eCBs then diffuse back across the synaptic cleft and bind to CBRs (blue) on the presynaptic cell. This predominately leads to a suppression of $[Ca^{+}]$ influx but also an increase in $[K^{+}]$ efflux, thus hyperpolarising the presynaptic cell and inhibiting further neurotransmitter release.

**Table 1;** endocannabinergic tone effecter ligands. A brief overview of the CBR ligands discussed throughout this review, giving further background to their discovery and where appropriate their non-feeding effects.

Abbreviations: 2-AG, 2-archidonlyglycerol; $\Delta^8$THC, $\Delta^8$tetrahydrocannabinol; $\Delta^9$THC, $\Delta^9$tetrahydrocannabinol; $\Delta^9$THCA, $\Delta^9$tetrahydrocannbinolic acid; $\Delta^9$THCV, $\Delta^9$tetrahydrocannbivarin; AEA, anandamide; $CB_1R$, cannabinoid type 1 receptor; $CB_2R$, cannabinoid type 2 receptor; CBR, cannabinoid receptor; CNS, central nervous system; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; OEA, n-oleoylethanolamide; P-gp, p-glycoprotein; $VR_1$, transient receptor potential vanniliod 1; $TRPA_1$, transient receptor potential cation channel, subfamily A, member 1.