1   *Cannabis sativa* **and the endogenous cannabinoid system:**

2   **therapeutic potential for appetite regulation**

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

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1 **Abstract**

2 The herb *Cannabis sativ*a (*C. sativa*) has been used in China and on the Indian

3 subcontinent for thousands of years as a medicine. However, since it was brought to the

4 U.K. and then the rest of the western world in the late 19th century, its use has been a

5 source of controversy. Indeed, its psychotropic side effects are well reported but only

6 relatively recently has scientific endeavour begun to find valuable uses for either the

7 whole plant or its individual components. Here, we discuss evidence describing the

8 endocannabinoid system, its endogenous and exogenous ligands and their varied effects

9 on feeding cycles and meal patterns. Furthermore we also critically consider the

10 mounting evidence which suggests non-Δ^9tetrahydrocannabinol phytocannabinoids play

11 a vital role in *C. sativa*-induced feeding pattern changes. Indeed, given the wide range

12 of phytocannabinoids present in *C. sativa* and their equally wide range of intra-, inter-

13 and extra-cellular mechanisms of action, we demonstrate that non-

14 Δ^9tetrahydrocannabinol phytocannabinoids retain an important and, as yet, untapped

15 clinical potential.

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1 **Introduction**

2 For ~10,000 years, *Cannabis sativa* (*C. sativa*) has been used medicinally and

3 recreationally for its diverse pharmacological actions and psychotropic properties.

4 Typical actions include sedation, analgesia, hypothermia, catalepsy and euphoria which

5 are thought to arise mainly from cannabinoid type 1 receptor (CB_1R) activation

6 (Childers *et al.*, 1998; Little *et al.*, 1988). These effects have typically been ascribed to

7 the sixty (Howlett, 2002; Pertwee, 2008b) to eighty (Izzo *et al.*, 2009)

8 phytocannabinoids (pCBs) present in *C. sativa*. In more recent times, *C. sativa* has been

9 used less widely for medicinal purposes, due in part to the development of other

10 therapeutic agents with better side effect profiles (e.g. Tramer *et al.*, 2001). Indeed,

11 cannabinoid side-effects are known to include reduced motor coordination, tachycardia,

12 transient memory loss, disruption to the sense of time, cognitive impairments, anxiety

13 and a disruption of appetite regulation (Childers *et al.*, 1998; Dewey, 1986; Grant *et al.*,

14 2005; Hollister, 1986; Kirkham *et al.*, 2001b; Wilkinson *et al.*, 2003) (for a recent

15 review on the safety of *C. sativa*-based therapy see Wang *et al.*, 2008). However, the

16 relatively recent identification of the CB_1R and cannabinoid type 2 (CB_2R) receptors,

17 alongside the subsequent characterisation of the endocannabinoid (eCB) system (for a

18 recent review see Maccarrone, 2009), has driven a resurgence of interest in the potential

19 applications of cannabinoid-based therapy. Cannabinoid therapies have now been

20 investigated for a wide range of neurological and neurodegenerative disorders (Glass,

21 2001; Pryce *et al.*, 2003), both in terms of treatment (for a review see Consroe, 1998)

22 and/or symptom management (Davis *et al.*, 2007). One notable example is in the

23 treatment of multiple sclerosis (Pryce *et al.*, 2005) and its symptoms (eg. spasticity), for

1   which a cannabis-based compound (Sativex®) has recently been licensed in the U.K.,

2   Spain and Canada by GW Pharmaceuticals.

3   'Cannabinoid' is a well-known term used to describe the compounds isolated

4   from *C. Sativa*, the pCBs such as Δ^9tetrahydrocannabinol (Δ^9THC), cannabidiol (CBD)

5   and cannabinol (CBN). In addition to these, the term now also encompasses both

6   synthetic exogenous (e.g. SR141716A, a CB_1R specific antagonist/inverse agonist) and

7   endogenously produced ligands (the eCBs anandamide (AEA) and 2-arachidonyl

8   glycerol (2-AG)) at CB_1- and CB_2Rs (for an overview of the cannabinoid receptor

9   (CBR) ligands referred to in this review, see table 1). The development and

10   characterisation of such pharmacological tools has enabled significant progress to be

11   made into understanding the eCB system over the last 20 years (Onaivi *et al.*, 2002).

12   Δ^9THC, the main psychoactive component in *C. sativa,* was first isolated and

13   synthesised in 1964 by Gaoni *et al.* (Gaoni *et al.*, 1964a). However, it was not until

14   1988 that the first cannabinoid specific G-protein coupled receptor (GPCR; CB_1R) at

15   which it acts was characterised (Devane *et al.*, 1988; Matsuda *et al.*, 1990), lending

16   credence to the postulated existence of an eCB system (Howlett *et al.*, 1990) (for review

17   see Pertwee, 2005). A series of studies (Herkenham *et al.*, 1991; Herkenham *et al.*,

18   1990) identifying the widespread expression of this receptor in the brain further

19   supported this discovery. These findings were rapidly followed by the isolation and

20   structural determination of the first eCB ligand, AEA, in 1992 (Devane *et al.*, 1992), the

21   identification and characterisation of CB_2R expressed on immune cells, in 1993 (Munro

22   *et al.*, 1993), and the identification of a second eCB ligand, 2-AG, in 1995 (Mechoulam

23   *et al.*, 1995b; Sugiura *et al.*, 1995) (See Fig.1). Since then, significant academic and

24   commercial effort has been invested in characterising and exploiting the eCB system

1 (for example reviews see Basavarajappa, 2007a; Basavarajappa, 2007b; Bisogno *et al.*,

2 2005; Freund *et al.*, 2003).

3 As previously outlined, it is now apparent that the eCB system is physiologically

4 ubiquitous and linked to many pathophysiological states (de Fonseca, 2005), resulting in

5 its identification as a therapeutic target, as well as the publication of numerous reviews

6 regarding the potential benefits of cannabinoid based therapies (e.g. Agrawal *et al.*,

7 2009; Baker *et al.*, 2003; Glass, 2001; Goutopoulos *et al.*, 2002; Makriyannis *et al.*,

8 2005). This is particularly true in the field of body weight regulation and energy balance

9 (Yates *et al.*, 2009), where significant advances have been made in the development of

10 both anti-anorectic (e.g. in hepatitis C (Costiniuk *et al.*, 2008); in cancer (Walsh *et al.*,

11 2003); in human immunodeficiency virus (HIV) (Woolridge *et al.*, 2005)) and anti-

12 obesity (Van Gaal *et al.*, 2005) cannabinoid-based therapies. However, these advances

13 must be made with care (Grant *et al.*, 2005), and it is clear that further long-term studies

14 are warranted if the safety profiles of such therapies are to be fully understood (Wang *et*

15 *al.*, 2008). Indeed, the withdrawal of Acomplia (Rimonabant: SR141716A; see EMA,

16 2009 for details) from clinical use for the treatment of obesity due to serious cognitive

17 side effects, combined with the cessation of Phase III clinical trials for a second putative

18 anti-obesity drug, Taranabant (MK-0364; Clark, 2009), illustrates the difficulties of

19 manipulating such a complex and ubiquitous system. Nonetheless, it is clear that the

20 eCB system plays an important, if not yet fully understood, role in appetite, obesity and

21 energy balance (for reviews see Cota *et al.*, 2003a; Kirkham, 2008). This review will

22 describe the significant progress made, as well as the work still required, in order to

23 fully understand the role played by the eCB system in feeding and appetite regulation.

24 Importantly, by focusing on those studies that have examined pCB effects and how they

1   might interact with the eCB system to modulate appetite and feeding, this review

2   provides insight into the potential use of these compounds as therapeutic tools in the

3   treatment of eating- and weight-related disorders.

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

2 Although, CBRs are present in both the peripheral and central nervous systems (CNS),

3 the majority of cannabinoid feeding research to date has focused primarily on centrally-

4 mediated feeding mechanisms, a focus that is consequently reflected in this review. A

5 short section outlining the biological components that comprise the eCB system within

6 the CNS is presented hereafter (see also figures 2 and 3).

7 ECBs (AEA and 2-AG) are produced on demand by postsynaptic neurons (Di

8 Marzo *et al.*, 2004) in the CNS in response to rises in intracellular $[Ca^{2+}]$ (Cadas *et al.*,

9 1997; Di Marzo *et al.*, 1994). Such increases typically occur following G_q-coupled

10 receptor activation which stimulates production of inositol trisphosphate (IP_3) by

11 phospholipase C (PLC) leading to a release of Ca^{2+} from intracellular stores (for

12 reviews see Berridge, 1995; Berridge *et al.*, 1988; Putney Jr, 1986). Alternatively,

13 ionotropic glutamate receptor activation permits the influx of Ca^{2+} to increase eCB

14 production (Freund *et al.*, 2003).

15 AEA synthesis from its precursors, arachidonic acid and N-arachidonoyl

16 phosphatidyl-ethanolamine (*N*aPE) is an on demand effect. Phosphatidylethanolamine is

17 first catalysed by Ca^{2+}-dependent N-acyltransferase (Cadas *et al.*, 1997) into *N*aPE

18 (although Ca^{2+}-independent processes have also been proposed; Jin *et al.*, 2007); *N*aPE

19 is then converted to the N-acylethanolamine, AEA, (Di Marzo *et al.*, 1994) by *N*aPE

20 phospholipase-D (Okamoto *et al.*, 2004). However, it has been suggested that at least

21 three other pathways for AEA synthesis may also exist (for reviews, see Liu *et al.*,

22 2008; Okamoto *et al.*, 2009a; Okamoto *et al.*, 2009b). 2-AG is formed by the

23 diacylglycerol (DAG) lipases α and β-catalyzed hydrolysis of DAG (Di Marzo *et al.*,

24 2007). After formation, eCB release into the extracellular space is thought to occur

1 immediately via passive diffusion or through a putative eCB transporter as no storage

2 systems for eCBs have yet been identified and some eCB precursors are known to be

3 membrane bound, suggesting that eCBs are produced at their release site (Di Marzo,

4 2008; Pope *et al*, 2010.).

5 Once released into the intercellular space, unlike classical neurotransmission,

6 eCB signalling is retrograde such that eCBs released from a postsynaptic neuron diffuse

7 across the synaptic cleft to bind with presynaptically located CB_1Rs to exert effects (see

8 figure 3; Ohno-Shosaku *et al.*, 2001; Piomelli *et al.*, 2000; Wilson *et al.*, 2001) that are

9 now thought to be intimately linked to synaptic plasticity (see Alger, 2009). The

10 activation of presynaptic CB_1Rs by AEA and 2-AG induces a PLC-β-mediated

11 suppression of $[Ca^{2+}]$ entry and a protein kinase A (PKA)-mediated upregulation of $[K^+]$

12 efflux, thereby driving neuronal resting membrane potential to more hyperpolarized

13 values and so reducing the further pre-synaptic release of neurotransmitter (for review

14 see Mackie, 2006; Südhof *et al.*, 2008). Indeed, presynaptically it has been conclusively

15 demonstrated that CB_1R activation has a direct effect on membrane polarisation, for

16 example, in cultured hippocampal neurones (Deadwyler *et al.*, 1995; Schweitzer, 2000)

17 and in CA1 pyramidal cells (Kirby *et al.*, 2000).

18 ECB reuptake however remains the subject of much debate. Fatty acid amide

19 hydrolase (FAAH; Cravatt *et al.*, 1996) and monoacylglycerol lipase (MAGL; Lambert

20 *et al.*, 2005) are the two main enzymes involved in eCB degradation, and have been

21 known to degrade AEA and 2-AG for some time. However, although a specific

22 transmembrane transporter protein has yet to be identified, it seems that reuptake may

23 be a rapid carrier-mediated transport process (e.g. Gerdeman *et al.*, 2002; Ronesi *et al.*,

24 2004) since eCBs within the synaptic cleft are almost immediately inactivated (Di

1 Marzo *et al.*, 2007). 2-AG is typically inactivated by MAGL (Di Marzo, 2008) but also

2 to some extent by FAAH (Blankman *et al.*, 2007), whereas, AEA is thought to be

3 degraded by FAAH alone (Vandevoorde, 2008). Four mechanisms are currently

4 proposed to underlie eCB reuptake; a membrane-localised transmembrane carrier

5 protein, FAAH-induced passive diffusion through the lipid bilayer, the creation of

6 caveolae with AEA binding sites on the cell membrane which then internalise AEA

7 and/or 2-AG, and via intracellular sequestration into the cell within a lipid shell (for a

8 recent review see Yates *et al.*, 2009). Intracellular FAAH-mediated AEA hydrolysis

9 creates arachidonic acid and ethanolamine (Deutsch *et al.*, 2002 thoroughly review the

10 biochemistry of FAAH) whilst 2-AG undergoes primarily MAGL-mediated hydrolysis

11 into arachidonic acid and glycerol (Blankman *et al.*, 2007).

12 While the mechanisms involved in AEA and 2-AG degradation remain poorly

13 understood, it is clear that FAAH and MAGL blockade could be of therapeutic value.

14 FAAH inhibition, for instance by URB597 (Piomelli *et al.*, 2006; see Vandevoorde,

15 2008 for a review of FAAH and MAGL inhibitors), is expected to potentiate AEA

16 effects (and to some extent 2-AG). Such inhibition could, for instance, be expected to

17 have effects on energy homeostasis as AEA and 2-AG administration into the

18 hypothalamic nuclei has been conclusively demonstrated to induce hyperphagia in rats

19 (Jamshidi *et al.*, 2001; Kirkham *et al.*, 2001a; Kirkham *et al.*, 2002). Consequently,

20 increasing AEA concentrations in the synaptic cleft via FAAH inhibition could produce

21 similar effects. Although MAGL inhibition (e.g. by URB602; King *et al.*, 2007) which

22 could potentiate 2-AG effects is less well studied than FAAH inhibition, it too could be

23 of comparable therapeutic use. It is however important to consider that both FAAH and

24 MAGL inhibition could have secondary effects besides increasing AEA and 2-AG

1   levels respectively. For instance, some evidence suggests that FAAH inhibition can also

2   decrease 2-AG levels (see Di Marzo *et al.*, 2008 for a review of these data), and MAGL

3   inhibition, by the particularly potent and selective MAGL inhibitor JZL-184, has been

4   found to induce behavioural effects similar to those produced by agonist-induced CB_1R

5   activation (Long *et al.*, 2009). The dynamic interplay between these systems must be

6   taken into account when considering new therapeutic approaches if unwanted side-

7   effects are to be minimised.

8   The cannabinoid receptors, CB_1 and CB_2R, are members of the superfamily of

9   GPCRs and are characterised by seven transmembrane helices (Turu *et al.*, 2009). CB_1R

10   was cloned in 1990 (Matsuda *et al.*, 1990), 26 years after the isolation of Δ^9THC.

11   CB_2Rs were first identified and characterised via polymerised chain reaction

12   experiments in 1993 (Munro *et al.*, 1993; Onaivi *et al.*, 2006), and were initially thought

13   to be present only in inflammatory/immune cells; Basavarajappa, 2007b; Munro *et al.*,

14   1993), although evidence now shows CB_2R expression in the brain (Gong *et al.*, 2006;

15   Onaivi *et al.*, 2006; Van Sickle *et al.*, 2005). Indeed, it is now known that both CB_1- and

16   CB_2Rs are widely expressed in the brain (Gong *et al.*, 2006; Moldrich *et al.*, 2000)

17   although it is postulated that the majority of the functional eCB effects in the CNS are

18   CB_1R mediated (Piomelli, 2003). This receptor is one of the most abundant GPCRs in

19   the CNS (Herkenham *et al.*, 1991) and its distribution within the mammalian brain

20   reflects the behavioural effects of cannabinoid administration. For instance, CB_1R levels

21   are particularly high in the cortex, hippocampus, cerebellum and basal ganglia,

22   correlating with the well-known effects of cannabinoids on cognition, memory and

23   motor control respectively. (Herkenham *et al.*, 1991; Tsou *et al.*, 1997; for a detailed

24   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 Δ^9tetrahydrocannabivarin; Δ^9THCV) 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

1   see Sanger, 2007), is currently a putative third CBR. Upon activation, GPR55

2   suppresses M-type K^+ currents and stimulates phosphatidylinositol 4,5-bisphosphate

3   (PIP2) -induced Ca^{2+} release from intracellular stores (Henstridge *et al.*, 2009; Lauckner

4   *et al.*, 2008). While the status of GPR55 as a CBR has been a topic of fierce academic

5   debate for some years (Petitet *et al.*, 2006), somewhat more compelling evidence has

6   been presented recently suggesting that it is indeed a CBR (Baker *et al.*, 2006;

7   Henstridge *et al.*, 2010; Kapur *et al.*, 2009; Lauckner *et al.*, 2005; Pertwee, 2007; Ross,

8   2009; Ryberg *et al.*, 2007; Staton *et al.*, 2008). Both Baker (Baker *et al.*, 2006) and

9   Ryberg (using radioligand binding assays in human embryonic kidney (HEK293) cells;

10   Ryberg *et al.*, 2007) have shown that CP55,940 (a non-selective CBR agonist), but not

11   WIN55212-2 (a CBR agonist), binds with strong affinity to GPR55; Baker also showed

12   that AEA, 2-AG and Δ^9THC bind to this receptor. Moreover, Ryberg presented further

13   evidence in the same manuscript suggesting that GPR55 was $G_{\alpha 13}$ coupled, and that the

14   comparatively late detection of GPR55 as a possible CBR was because the affinity of

15   CP55940 for GPR55 binding was 25 times lower than that for CB_1R binding. However,

16   in a similar study Lauckner and colleagues (Lauckner *et al.*, 2005) found that while

17   Δ^9THC could activate GPR55 in dorsal root ganglia (DRG) cells, neither WIN55212-2

18   nor CP55,940 bound to GPR55 in HEK293 and Chinese Hamster Ovary (CHO) cells.

19   Furthermore the observed effects were blocked by application of SR141716A, a CB_1R

20   specific antagonist, (although not by SR144528, a CB_2R specific antagonist), suggesting

21   that these were CB_1R rather than GPR55 mediated. The reasons behind the difference in

22   CP55,940 binding between the studies by Baker, Ryberg and Lauckner are not

23   immediately apparent, although Lauckner *et al.* suggest it may be due to a difference in

24   GPR55 transfection between the cell types used. Despite such discrepancies, however,

1   evidence is mounting for GPR55's role in the eCB system, as demonstrated for instance

2   by Kapur and colleagues, who observed AM251 (CB_1R inverse agonist) and

3   SR141716A (a CB_1R specific antagonist) binding to GPR55 (Kapur *et al.*, 2009).

4   Whilst it clearly remains an 'enigmatic' receptor (Ross, 2009), research is beginning to

5   focus on possible GPR55 based therapies, with recent studies linking it to

6   neuroinflammation (Pietr *et al.*, 2009), inflammatory and neuropathic pain (Staton *et al.*,

7   2008) and anorexia (Ishiguro *et al.*, 2010).

8   Finally, although the identification of GPR119 in 2003 did not initially suggest

9   links to the eCB system (Fredriksson *et al.*, 2003), later work by Overton and Soga

10   showing N-oleoylethanolamide (OEA), an AEA analogue, induced GPR119 activation

11   (Overton *et al.*, 2006; Soga *et al.*, 2005) suggested otherwise. Soga *et al.* and more

12   recently Ning *et al.* observed that GPR119 agonism mediates glucose-dependent insulin

13   production and release to some extent, as well as increasing intercellular cyclic

14   adenosine monophosphate (cAMP) accumulation (Ning *et al.*, 2008; Soga *et al.*, 2005).

15   However, despite findings that GPR119 agonism by OEA suppresses feeding in rats

16   (Rodriguez de Fonseca *et al.*, 2001), this receptor is unlikely to be involved in centrally

17   mediated eCB feeding effects as it has been found in the gut (Overton *et al.*, 2006;

18   Overton *et al.*, 2008). It should nevertheless still be considered when interpreting *in vivo*

19   feeding study results and could represent a useful target for anti-obesity therapies

20   (Hughes, 2009).

21   Although, as previously mentioned, this review focuses primarily on central

22   cannabinoid mediated feeding mechanisms, peripheral effects, such as those mediated

23   by peripheral CBRs or GPR119 add considerable complexity when interpreting *in vivo*

24   feeding data. This is particularly true in light of findings describing peripheral CB_1R

1 expression e.g. in the vas deferens: Pertwee *et al.*, 1996; in the heart, blood vessels and

2 bladder: Howlett, 2002; the liver and fatty tissue: Cota *et al.*, 2003b; Osei-Hyiaman *et*

3 *al.*, 2005), and a potential modulatory role of these peripheral receptors on feeding

4 behaviour (Gomez *et al.*, 2002). Equally, although it is thought that most of the

5 functional eCB effects in the brain are CB_1R-mediated (Piomelli, 2003), recent work

6 has suggested some functional expression of CB_2Rs in the brain (Onaivi *et al.*, 2006),

7 particularly at synapses (Morgan *et al.*, 2009), and that these may also be involved in

8 feeding behaviours (Onaivi *et al.*, 2008). Furthermore, CBR activation in the gut for

9 example, in terms of gut motility (Izzo *et al.*, 2010), are also likely to have considerable

10 effects on feeding patterns. As such, it is often difficult, when interpreting *in vivo*

11 experimental results, to exclusively assign cannabinoid effects to either central or

12 peripheral sites of action, or to differentiate between CB_1- and CB_2-, or indeed non-

13 CBR-mediated effects. Moreover, ligands which produce well-characterised effects at

14 specific CBRs may also exert effects at other receptor sites, thereby creating unexpected

15 and potentially unwanted secondary effects. This is well illustrated by SR141716A

16 (Acomplia/Rimonabant), a well-known selective CB_1R antagonist/inverse agonist which

17 has recently been found by Kapur *et al.* to agonise GPR55, and which the authors

18 suggest may have played a part in some of the 'off-target' effects that led to its

19 withdrawal as an anti-obesity drug (Kapur *et al.*, 2009).

20   There is still much to clarify about how cannabinoids produce their endogenous

21 effects, and how they might interact with each other *in vivo* (Ben Amar, 2006; Whalley

22 *et al.*, 2004). Furthermore, adding to this complexity is the eCB system's interaction

23 with other systems, in particular the opioid system which itself has been linked to

24 appetite regulation for some time (for an early review of opiates and appetite regulation

1   see Morley *et al.*, 1982). Indeed, it is now apparent that both the eCB and opioid

2   systems modulate energy balance, that both systems can modulate food intake

3   independently and that activation or suppression of both systems together can increase

4   or reduce food intake to a greater extent than modulation of either system alone (Gallate

5   *et al.*, 1999; Kirkham *et al.*, 2001c; Solinas *et al.*, 2005). Whilst detailed discussion of

6   such interactions is beyond the scope of this review, they, along with the numerous

7   other complexities outlined above, are important factors to consider when looking at, or

8   indeed conducting and analysing, research on the eCB system and feeding.

9

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1   **Cannabinoid-mediated effects upon feeding**

2   The last decade has seen considerable advances in our understanding of eCB-mediated

3   control of feeding behaviours. Numerous anecdotal reports exist, and the 'munchies'

4   effect has become a well-accepted physiological response to *C. sativa* consumption, yet

5   few quantitative reports from this period exist (for a complete review see Kirkham *et*

6   *al.*, 2001b). While the 'classical' tetrad of cannabinoid effects was defined as decreased

7   spontaneous motor activity, hypothermia, analgesia and catalepsy (Little *et al.*, 1988), it

8   was only comparatively recently that Δ^9THC was demonstrated to induce hyperphagia

9   (Williams *et al.*, 1998), an effect clearly linked to CB_1R rather than CB_2R activation

10   (Williams *et al.*, 2002b). Since CB_2R have only recently been localised to the CNS

11   (Morgan *et al.*, 2009), and have not been found expressed on feeding pathways, little

12   research has considered the possible effects that CB_2R stimulation may have on feeding.

13   While a limited number of reports which consider CB_2R effects on feeding do exist (e.g.

14   Onaivi *et al.*, 2008), they have so far presented inconclusive evidence. Furthermore,

15   possible opioid/cannabinoid and cannabinoid/serotonergic interactions have been

16   studied, for example by Williams *et al.,* (2002b). Williams demonstrated a functional

17   relationship between the cannabinoid and opioid systems in the control of appetite, and

18   notably rejected any serotonergic interaction: upon administration of Δ^9THC and either

19   SR141716A, SR144528, naloxone or dexfenfluramine, Williams observed that while

20   naloxone could block Δ^9THC-mediated feeding increases, dexfenfluramine could not.

21   Unfortunately, a detailed description of such interactions falls beyond the scope of this

22   review, although a recent review of the effects of CB/opioid interactions on the

23   behavioural satiety sequence can be found in Cota *et al.* (2006). Given the relatively

24   recent success of treatment with Δ^9THC-based CB_1R partial agonists (e.g. dronabinol or

1   nabilone) and the remaining unmet clinical need in a range of disease states, further

2   research into pCB-mediated feeding effects is on-going and is reviewed here alongside

3   classical CB_1R agonism and antagonism.

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1   *Endocannabinoid agonism and its effects on feeding*

2   Δ^9THC was first shown to exert hyperphagic effects in rats in 1998 (Williams *et al.*,

3   1998). Δ^9THC (0.063-2.0mg/kg) administered orally (per ora; p.o.) to pre-satiated (a

4   situation in which eCB tone is reduced by the highly palatable prefeed process), adult,

5   male, Lister hooded rats, at doses >0.5mg/kg significantly increased chow intake

6   relative to control in the first hour following drug administration. Furthermore, no

7   significant differences in food intake between control and any Δ^9THC doses were seen

8   over the twenty four hour period following Δ^9THC administration. This early study

9   importantly demonstrated that very low concentrations of orally administered Δ^9THC

10   could substantially change an animal's motivation to consume; particularly given that a

11   considerable percentage of the administered drug would have undergone first pass

12   metabolism as a result of oral administration. Intriguingly, a later study by Avraham

13   (Avraham *et al.*, 2004) also showed that extremely low Δ^8THC doses (a pCB similar to

14   Δ^9THC but regarded as more stable; 0.001mg/kg; intraperitoneal; i.p.) increased feeding

15   in mice. These studies suggest that subtle modulation of the eCB system can be

16   achieved without the manifestation of the non-specific behavioural side effects

17   traditionally associated with *C. sativa* administration (Howlett *et al.*, 2004). The drug

18   administration routes used by Williams (1998; p.o.) and Avraham (i.p.) do, however,

19   limit comparison of these results and prevent specific attribution of the effects to central

20   or peripheral sites.

21   Exogenous AEA administration (0.5, 1.0, 5.0 and 10.0mg/kg; subcutaneous;

22   s.c.) has also been observed to induce hyperphagia in pre-satiated rats (Williams *et al.*,

23   1999). This study demonstrated that AEA administration significantly increased food

24   intake relative to control in the first hour of testing, in a similar fashion to Δ^9THC

1 (Williams *et al.*, 1998), but unlike Δ^9THC also induced increases in total food intake. It

2 was proposed that the observed changes in food intake pattern represented an AEA-

3 induced modification in the *motivation* to feed, since AEA administration caused

4 feeding to occur far sooner than under control conditions, suggesting a cannabinoid-

5 mediated modulation of normal feeding pathways. Indeed, close inspection of these data

6 shows that while vehicle-treated animals consumed only small amounts of food in the

7 first hour of testing, they consumed ten-fold more during the third hour. In slightly later

8 work, Williams *et al.* (2002a) used two groups of twelve, male, adult, Lister-hooded rats

9 which received Δ^9THC (0.5, 1.0 or 2.0mg/kg; s.c.) or AEA (0.0, 1.0, 5.0 or 10.0mg/kg;

10 s.c.) and video recordings of post-administration activity to investigate feeding

11 behaviours. It became clear that administration of both Δ^9THC and AEA significantly

12 decrease the latency to feeding onset, increase the duration of intake and the number of

13 meals, but that only AEA increased total intake. Alongside the well-reported increases

14 in short term chow intake associated with Δ^9THC, these data suggest that Δ^9THC and

15 AEA administration induce their effects by subtly different, and yet to be elucidated

16 pathways since two distinct changes to feeding behaviours were observed.

17 Like Δ^9THC, extremely low doses of AEA (0.001mg/kg; i.p.) have also been

18 demonstrated to induce significant increases in food intake in female mice in

19 experiments which used a 40% diet restriction protocol (Hao *et al.*, 2000). It is

20 interesting that in a situation when eCB tone would already be increased due to the diet

21 restriction paradigm used, further slight increases in eCB tone caused by extremely low

22 doses of AEA induced changes in feeding patterns. Furthermore, it is also interesting

23 that when Hao and colleagues administered doses of 0.7 and 4.0mg/kg AEA (also i.p.)

24 in the same set of experiments no significant feeding effects were observed, even

1   though, Williams (1999) demonstrated AEA effects at 1.0 and 5.0mg/kg (s.c.).

2   However, as the authors note, it is particularly difficult to compare Williams *et al.*

3   (1999) and Williams *et al.* (2002a) to Hao *et al.* (2000) since the experimental

4   paradigms are almost complete opposites. Indeed, Williams used a prefeed paradigm

5   followed by s.c. injections in male rats and recorded food intake over a period of six

6   hours whereas Hao used a diet restricted paradigm followed by i.p. injections in female

7   mice and recorded daily food intake over a period of one week.

8   AEA feeding effects were localised to CB_1R sites by Williams using a pre-

9   satiated paradigm in 1999. Rats received AEA (0.5, 1.0, 5.0 and 10.0mg/kg; s.c.) and, in

10   a subsequent trial, AEA (1.0mg/kg; s.c.) plus SR141716A (0.1, 0.5 and 1.0mg/kg; s.c.;

11   Williams *et al.*, 1999). This demonstrated that AEA significantly increased food intake

12   relative to control (in a similar fashion to Δ^9THC; Williams *et al.*, 1998) and that the

13   effect could be blocked by SR141716A, thus confirming a CB_1R-mediated mechanism

14   of action for the observed feeding effects. In 2002 the hyperphagia induced by Δ^9THC

15   administration was also localised to CB_1R sites (Williams *et al.*, 2002b). Williams first

16   induced hyperphagia by administering Δ^9THC (1.0mg/kg; s.c.) and in further trials

17   attenuated this effect with the coadministration of SR141716A (0.1, 0.5 and 1.0mg/kg;

18   s.c.) thereby demonstrating CB_1R involvement in this effect. CB_2R-mediation was then

19   discounted as coadministration of Δ^9THC (1.0mg/kg; s.c.) and SR144528 (0, 0.05, 0.1,

20   0.5 and 1.0mg/kg; s.c.) failed to reduce the hyperphagia caused by Δ^9THC

21   administration.

22   Although Williams *et al.* (1999) localised AEA-mediated effects on feeding to

23   CB_1R, an effect which one can attribute to CNS receptors, evidence which links

24   peripheral mechanisms to these effects also exists. Gomez and colleagues (Gomez *et al.*,

1   2002) demonstrated that intracerebroventricular (i.c.v.) AEA administration (10.0μg/kg)

2   and WIN55,212 (10.0μg/kg) increased food intake, but that i.c.v. SR141716A

3   administration (0.1, 0.4, 2.0 or 10.0μg/kg) did not reduce feeding. In contrast, whilst i.p.

4   administration of AEA (0.1, 1.0 and 10.0mg/kg) and WIN55,212 (0.4, 2.0 and

5   10.0mg/kg) similarly increased food intake, i.p. administration of SR141716A (0.3, 1.0

6   and 3.0mg/kg) reduced feeding. Furthermore, the authors demonstrated that the changes

7   in feeding patterns they observed were unaffected by a CB_2R antagonist (SR144528;

8   3.0mg/kg; i.p.), principally rejecting the hypothesis that these peripheral effects were in

9   fact CB_2R- as opposed to CB_1R-mediated. SR141716A's failure to reduce feeding after

10   i.c.v. but not i.p. administration led Gomez to suggest that CB_1Rs must be present in the

11   periphery and that these peripheral CB_1Rs can affect feeding. Gomez supports this

12   assertion by showing that intestinal AEA levels increase after food deprivation,

13   suggesting that intestinal endocannabinergic tone plays a role in energy balance.

14   However, the experimental paradigm used by Gomez did not account for the

15   concentrations of SR141716A, AEA and WIN55,212 that would be present in the CNS

16   following i.p. injection or for the considerably different dose ranges used. As such,

17   while this evidence is suggestive of a peripheral CB_1R-mediated role in energy balance,

18   further experiments are needed to confirm this.

19   It has been well described that upon administration of an exogenous cannabinoid

20   agonist endocannabinergic tone will be increased and that alterations to feeding patterns

21   can then be observed. Furthermore, it has been demonstrated that naturally occurring

22   eCB concentrations fluctuate in various feeding states. Alongside evidence gathered

23   using exogenous cannabinoid administration to affect feeding patterns, this elegantly

24   demonstrates a link between how the natural eCB system modulates feeding patterns

1   and how artificial alterations to its state modulate the same patterns. AEA and 2-AG

2   levels in the rat limbic forebrain, hypothalamus and cerebellum were quantified in three

3   feeding states; fasted, feeding and satiated (Kirkham *et al.*, 2002). Four groups of

4   between eight and ten male rats were either: 1) given *ad libitum* access to food and

5   sacrificed during a period of low spontaneous feeding; 2) fed with a wet mash for 15

6   minutes and then sacrificed during feed consumption; 3) fed with a wet mash and

7   sacrificed once they stopped eating or 4) given a 20% maintenance diet for 24hrs and

8   sacrificed at the start of their red light cycle the following day. Brain tissue was then

9   extracted and AEA and 2-AG levels were assessed in the limbic forebrain,

10   hypothalamus and cerebellum. AEA and 2-AG levels were significantly elevated by

11   food deprivation in the limbic forebrain while 2-AG concentration was significantly

12   reduced in the hypothalamus during the feeding state but significantly increased during

13   the deprived state. Since 2-AG levels were increased in the limbic forebrain and

14   hypothalamus, and AEA levels were significantly increased in the limbic forebrain, it

15   appears that elevated eCB levels in important reward-related brain areas during food

16   deprivation play a role in motivating animals towards food. The reduction of 2-AG

17   levels in the hypothalamus during feeding suggests that 2-AG is actively suppressed

18   during feeding to facilitate satiation. Such data suggest subtly differing roles for AEA

19   and 2-AG in feeding cycles.

20   Further experiments published in the same manuscript revealed that 2-AG (i.c.v.

21   into the nucleus accumbens at 0.5 and 2.0μg) could significantly increase food intake

22   over a one hour period while coadministration of SR141716A (0.5mg/kg; s.c. plus

23   0.5μg 2-AG; i.c.v.) could almost fully attenuate this effect, thereby demonstrating CB_1R

24   mediation. The increases in food intake seen in Kirkham *et al.* (2002) are comparable to

1 the similar studies performed by the same group following Δ^9THC administration

2 (Williams *et al.*, 2002b; Williams *et al.*, 1998), however are considerably different from

3 a feeding study which administered AEA (Williams *et al.*, 1999). Indeed, AEA

4 (1.0mg/kg; s.c.) effects were observed over four hours and during the first hour

5 significantly increased intake by a factor of approximately two, as well as significantly

6 elevating food intake over the entire four hour period. In contrast, 2-AG (0.5 and

7 1.0μg/kg; i.c.v.) significantly increased food intake by factors of nine and seven,

8 respectively, over a one hour period. However, it is important to note that in this

9 instance 2-AG administration was i.c.v. and AEA administration was s.c., and it is

10 possible that AEA-mediated feeding increases would have been larger had

11 administration been i.c.v. Given the strong anatomical connections between the nucleus

12 accumbens shell and the hypothalamus (Stratford *et al.*, 1999) and the importance of the

13 hypothalamus in the integration of feeding pathways (Maccarrone *et al.*, 2010) these

14 data represent a demonstration that modulation of this pathway can have profound

15 feeding effects.

16   As considerable differences in eCB levels in the limbic forebrain were seen

17 during various feeding states in (Kirkham *et al.*, 2002) and because the limbic forebrain

18 is traditionally associated with reward processing, possible interactions between the

19 eCB and opioid systems were then considered by Williams and Kirkham (Williams *et*

20 *al.*, 2002b). It was demonstrated that Δ^9THC-induced hyperphagia could be attenuated

21 by the opioid antagonist naloxone. This proves a link between opioid food reward

22 processing and the eCB system. These experiments are also exciting in terms of putative

23 anti-obesity treatments. Indeed, it has been reported that in obese humans, eCB levels

24 are elevated by up to 52% (Engeli *et al.*, 2005); as such experiments which artificially

1   elevate eCB activity using food-restriction paradigms are particularly valuable since

2   they more accurately model the human obese condition. Moreover, since in both the

3   food deprived and obese states eCB levels are increased, this further supports the theory

4   that eCB dysfunction plays an important part in human obesity. Further studies which

5   directly compared AEA and 2-AG-induced feeding effects (both centrally and

6   peripherally administered) would be extremely useful. It would be equally compelling

7   for further work to consider fully the putative effects of peripheral cannabinoid

8   receptors on feeding behaviours, for example in terms of gut motility.

9   Given the previously discussed alterations to eCB concentrations in the limbic

10 forebrain, an area traditionally associated with reward processing and well documented

11 opioid/cannabinoid interactions, it is clear that the changes to behavioural patterns

12 observed during cannabinoid administration are due to changes in reward levels.

13 Therefore, cannabinoid agonist effects on the consumption of different types of

14 rewarding ingesta, such as sweet versus fatty foods, was addressed in a study by Koch

15 (2001). Here, Δ^9THC (0.0, 0.5, 1.0 and 2.5mg/kg; i.p.) was administered to rats

16 receiving either control (chow), high fat (HF) or high fat plus sugar (HFS) diets and

17 food intake was recorded over 24 hours post-injection. After the first hour, 0.5 and

18 1.0mg/kg Δ^9THC significantly increased food intake versus control within the HF diet

19 group only, while doses of 2.5mg/kg had no significant effects on intake in any group.

20 However, overall food intake during the first hour was significantly greater in the HF

21 group than in either the control chow or HFS groups. During the second hour of testing,

22 the 0.5 and 1.0mg/kg Δ^9THC doses significantly increased intake versus control in all

23 three groups with 1.0mg/kg causing the largest increases. This dose also induced a

24 significantly greater intake increase in the HF (but not in the HFS) than in the control

1 chow group, although overall food intake across all doses was significantly greater in

2 both the HF and HFS groups, relative to the control chow group. Finally in the fourth

3 hour, unlike previous feeding studies employing Δ^9THC which showed effects lasting

4 fewer than four hours, Δ^9THC doses of 1.0mg/kg still induced significant increases in

5 consumption compared to vehicle in the HF and HFS diet groups.

6   The increases in intake seen at lower dose levels by Koch appear comparable

7 (though reduced) to those previously reported (Williams *et al.*, 1998) and support a

8 cannabinoid-mediated stimulation of intake (Williams *et al.*, 1999; Williams *et al.*,

9 1998), although the non-significant effects of 2.5mg/kg Δ^9THC remain unexplained,

10 particularly as no changes to motor coordination or the appearance of tetrad effects were

11 reported for this dose. Therefore, it is clear from these results that eCB system

12 stimulation induces greater increases in the intake of highly fatty food-types (HF) than

13 other, less calorific or more palatable, diets (HFS). Previous work by Arnone *et al.*

14 (1997) and Simiand *et al.* (1998) (this work is fully discussed in 'Endocannabinoid

15 antagonists and their effects on feeding') would suggest that the HFS intake should also

16 have been increased by Δ^9THC administration. Indeed, Δ^9THC administration induced

17 similar intake increases in the HFS and HF diet groups but only after the fourth and

18 twenty-fourth hours of testing. These data could suggest that the eCB system is

19 involved more strongly in increasing the motivation to consume high fat foods and that

20 this effect manifests more quickly than eCB increases due to other 'bland' food types.

21 From the perspective of evolution, an increased motivation to consume high fat foods is

22 obvious and as such it is reasonable to suggest from this data that the eCB system is

23 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 Δ^9THC 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 Δ^9THC 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),

1   Gallate *et al.* (1999) and Williams *et al.* (1999) it can be suggested that eCB system

2   stimulation upregulates the palatability of high calorie, low palatability foods and

3   conversely, reducing endocannabinergic tone diminishes perceived palatability. This

4   could explain why in Koch's work the HF diet intake was increased above HFS diet as

5   it was both palatable and calorie-rich.

6   In a very recent study Yoshida and colleagues (2010) administered AEA and 2-

7   AG to both wild type and CB_1R knock-out mice. They observed that AEA and 2-AG

8   preferentially increased taste responses to sweet rather than salty, sour, bitter or umami

9   (a Japanese term for a meaty or savoury taste) flavours in the chorda tympani nerve,

10   fungiform taste buds and during a short-term lick response test. Furthermore, they did

11   not observe this effect in their knock-out mice, hence linking this effect to CB_1R. Given

12   their i.p. route of administration, the authors have suggested a peripheral effect,

13   alongside the well described central effect, of eCBs on taste. In comparison with the

14   work of Higgs *et al* (2003) who found 2-AG to have little effect on palatability in a

15   progressive lick-based paradigm, and considering that both studies used i.p.

16   administration and observed peripheral taste responses, it is not immediately apparent

17   why different eCBs are identified as the principal modulators of taste.

18   In earlier taste reactivity studies, Gallate *et al.* (1999) presented rats with various

19   concentrations of 'beer' (<0.5%, 2.7% or 4.0% ethanol solutions) or an 8.6% sugar

20   solution, and their motivation to consume these substances was measured using a lick-

21   based progressive ratio paradigm. In order to assess CB_1R involvement, animals were

22   treated with either vehicle, the CB_1R agonist CP55,940 (10.0, 30.0 or 50.0μg/kg; i.p.),

23   CP55,940 (30.0μg/kg; i.p.) plus SR141716A (1.5mg/kg; i.p.), or naloxone (2.5mg/kg;

24   i.p.) plus CP55,940 (30.0μg/kg; i.p.). This drug regime therefore also examined

1   potential interactions between the cannabinoid and opioid systems. In agreement with

2   previous research, CP55,940-treated animals exhibited a significant trend whereby

3   increasing dose led to increases in both beer and sucrose solution consumption.

4   SR141716A-induced CB_1R blockade during CP55,940 treatment attenuated this effect,

5   reducing beer solution intake to levels that did not differ from vehicle treatments.

6   Furthermore, CP55,940 plus naloxone administration significantly reduced beer intake

7   to approximately 50% of vehicle intake, confirming a strong interaction between CB_1

8   and opioid receptors in the control of alcohol consumption. This study demonstrated

9   that CB_1R activation could induce significant increases in palatable solution intake

10   when compared to vehicle-treatments, and that this effect was attenuated by CB_1R

11   antagonists; further supporting the theory of CB_1R involvement in perceived

12   palatability.

13   In 2005 and 2007 Jarrett *et al.* (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007)

14   demonstrated that the eCB system mediated the perceived palatability of sweet and

15   bitter tastes. In trials using adult, male Sprague-Dawley rats, Δ^9THC (0.5mg/kg; i.p.),

16   Δ^9THC (0.5mg/kg; i.p.) plus SR141716A (2.5mg/kg; i.p.) or AM251 (1.0mg/kg; i.p.), or

17   AM251 (1.0mg/kg; i.p.) alone were administered via intraoral cannulae and the

18   orofacial reactions to intraoral administration of either sucrose (2, 10 and 32%), or

19   quinine (0.01 or 0.05%) solutions were recorded. Δ^9THC increased the perceived

20   palatability of the sucrose solution, an effect attenuated in animals treated with Δ^9THC

21   plus SR141716A (Jarrett *et al.*, 2005). In the later study, Δ^9THC reduced aversiveness

22   to 0.05% quinine solution, an effect that was blocked by coadministration of AM251

23   (Jarrett *et al.,* 2007). The authors suggest that their results indicate that increases in

24   CB_1R responsiveness could be involved in a general upregulation of the ingesta

1   palatability. In further experiments during the same two studies, Jarrett and colleagues

2   also demonstrated that SR141716A administration alone does not reduce perceived

3   palatability (Jarrett *et al.*, 2005) but AM251 administration enhances aversiveness

4   (Jarrett *et al.*, 2007). This may suggest that the increased palatability of sweet food can

5   activate the eCB system and upregulate eCB tone. However, in the case of bitter tasting

6   food, when eCB tone would not be increased by the low palatability of the food, CB_1R

7   antagonism can then reduce the perceived palatability of the food further.

8   Further supporting this palatability hypothesis, it has recently been demonstrated

9   that, under a reinforced learning paradigm which included food type (normal lab chow,

10   chocolate pellets or fat-enriched pellets) selection as an experimental variable, mice will

11   select the sugar rich, highly-palatable food type or fatty foods over standard food after

12   Δ^9THC (1.0mg/kg; i.p.) administration (Barbano *et al.*, 2009). Since, the chocolate-

13   enhanced pellets used in this study had the same calorific content as the standard food

14   pellets also employed while the fat enriched pellets contained approximately 66% more

15   calories per gram but no added chocolate, it can be observed that Δ^9THC administration

16   increased the palatability of fatty foods to a level comparable to that of sweet foods.

17   The conclusion that the eCB system exerts a level of control over palatability

18   reward is well supported. It has been shown that increased eCB tone increases the

19   palatability of sweet liquids (Gallate *et al.*, 1999; Higgs *et al.*, 2003), bland fatty foods

20   (Koch, 2001) or even aversive, bitter liquids (Jarrett *et al.*, 2005; Jarrett *et al.*, 2007).

21   Indeed, eCB levels change naturally reflecting the current feeding state, and it is this

22   basal tone that can be altered with the application of CB_1R ligands. Furthermore, the

23   meal pattern changes induced by stimulation of the eCB system are also well

24   understood. Δ^9THC administration has been demonstrated to reduce the latency to

1   feeding onset in periods of reduced endocannabinergic tone and has also been shown to

2   increase meal sizes. If we consider appetitive behaviours as those that regulate the

3   latencies to meal onset and consummatory behaviours as those that regulate the size of

4   any given meal, what becomes clear from these data is that modulation of the eCB

5   system can affect consummatory *and/or* appetitive behaviours and that these different

6   behavioural changes can be manipulated individually. For example, in the work of

7   Williams *et al.* (2002a), a distinct separation was seen between the AEA and Δ^9THC

8   groups in terms of feeding behaviour. In this instance, AEA administration produced

9   effects comparable to Δ^9THC: AEA significantly reduced the latency to feeding onset,

10   whilst also increasing the number of eating bouts. AEA administration also increased

11   meal duration and the duration of the longest bout versus control conditions. However,

12   unlike Δ^9THC, AEA induced significant increases in total intake over the test period.

13   Given these data it can be clearly seen that the changes to feeding patterns (either in

14   gross terms or in terms of palatability levels) are due to changes to reward processing.

15   Indeed, the decreased latency to feeding onset and the increased size and length of

16   meals thereafter coupled with increased lick responses can all be linked to increased

17   reward.

18

19

20

1 *Endocannabinoid antagonism and its effect on feeding*

2 Alongside the well documented hyperphagic effects of Δ^9THC administration there is

3 comprehensive complimentary evidence which details the effects of reducing eCB tone

4 on feeding behaviours. Indeed the use of CB_1R antagonist-based studies represents an

5 elegant way to study the effects of basal eCB tone on physiological feeding

6 mechanisms. This is particularly true as many CBR antagonists are highly selective. In

7 1997 Arnone (Arnone *et al.*, 1997) and colleagues performed a comprehensive set of

8 experiments which revealed the changes to feeding behaviours induced by CB_1R

9 blockade by the administration of SR141716A (0.0, 0.1, 0.3, 1.0 or 3.0mg/kg: i.p.). In

10 experiments which studied either intake of sucrose pellets, intake of a 5% sucrose

11 solution or a 10% ethanol solution, Arnone demonstrated that eCB tone plays an

12 important role in the perceived appetitive values of both alcohol and sugar. Firstly,

13 Arnone and colleagues measured the weight of food consumed in 30min open field tests

14 where food-restricted male, Wistar rats (a situation in which one would expect eCB tone

15 to be increased by the food restriction (Kirkham *et al.*, 2002)) had access to either

16 sucrose pellets and standard lab chow or standard lab chow alone after SR141716A

17 administration. The quantity of sucrose pellets consumed by rats was reduced from

18 3.1±0.4g under control conditions to 1.0±0.4g after the 3.0mg/kg SR141716A dose in a

19 dose-dependent fashion; furthermore, the two highest doses of SR141716A induced

20 significant reductions when compared to control intakes. Standard chow intake was

21 unaffected. However, when Arnone repeatedly administered 3.0mg/kg SR141716A in

22 mice with a predisposition to alcohol consumption over four, six hour test periods in

23 one day, SR141716A induced a suppression of ethanol intake. Secondly, rats (male,

24 Sprague-Dawley) were given access to a 5% sucrose solution for a period of four hours

1   without access to food and water. As a control experiment another group of rats was

2   given access to water for the same test period. Sucrose solution intake was significantly

3   reduced by the two highest doses of SR141716A and a significant dose-dependent

4   reduction in sucrose solution intake was apparent. Water intake was unaffected by

5   SR141716A administration at any dose. Thirdly, Arnone tested the effect SR141716A

6   had on ethanol consumption in male C57BL6 mice; once again administration of either

7   1.0 or 3.0mg/kg SR141716A induced significant reductions in consumption versus

8   control-treatment and a significant dose-dependent effect was apparent for the entire

9   dose range. Water-only tests were used as a control, as per the 5% sucrose solution test,

10   and no effect of SR141716A was apparent. These three experiments demonstrate that

11   the eCB system plays an active role in the control of the appetitive value of sweet and

12   alcoholic ingesta. Indeed, such experiments prove that the eCB system must have a

13   constantly active basal tone as antagonists applied to an inactive system would induce

14   no behavioural changes alone. Furthermore, given the selectivity of SR141716A we can

15   be confident that this effect is localised to CB_1R sites.

16   Reductions in eCB tone induced by the administration of SR141716A (0.0, 1.0

17   and 3.0mg/kg; p.o.) were extended to marmosets in 1998 by Simiand (Simiand *et al.*,

18   1998). Marmosets were habituated to a high-sugar mash (HSM; 33% sugar, 67% milk

19   and cereal) and after habituation administered SR141716A twice a week; their food

20   (either HSM or standard diet) intake was then observed for a period of six hours.

21   Administration of the 3.0mg/kg dose induced significant reductions in HSM intake

22   versus control-treatments after one, three and six hours while the 1.0mg/kg dose only

23   reduced HSM intake versus control-treatments during hours one and three. Notably

24   standard diet intake was unaffected by SR141716A administration with the exception of

1   a significant increase in intake seen during hour six. These data demonstrate that

2   SR141716A can preferentially reduce intake of sweet, highly palatable food, versus

3   bland food. This suggests that eCB tone is responsible for increasing the appetitive

4   values of high-sugar foods under normal physiological conditions. Indeed, as previously

5   discussed, such work has been supported by later experiments which showed

6   SR141716A administration could reduce the reward associated with sucrose solutions

7   (Higgs *et al.*, 2003). Since Arnone *et al*. (1997) and Simiand *et al.* (1998), together,

8   used two strains of rats, one strain of mouse and one type of primate and observed

9   similar results, it can be suggested that the effects of the eCB system are similar across

10   species. However, the work of Arnone and Simiand did not consider a high fat diet

11   alternative to the high-sugar foods they used, and they only antagonised the eCB system

12   over a shorter period of time. It is worthwhile comparing the works of Arnone and

13   Simiand to that of Williams and colleagues. Indeed, while Williams observed CB_1R

14   stimulation to increase feeding, and that this effect could be blocked by

15   coadministration of SR141716A, Arnone and Simiand demonstrated a basal

16   endocannabinergic tone which when blocked with SR141716A alone could reduce

17   feeding. Furthermore, as discussed, the eCB system has been demonstrated to alter its

18   activity level dependent on feeding state (e.g. deprived of food versus eating; Kirkham

19   *et al.*, 2002). Taken together, these studies demonstrate that eCB tone is a powerful

20   effector of perceived hunger and that feeding reduces endocannabinergic tone in the

21   same way as blocking CB_1R sites in the CNS.

22   Δ^9THC-induced effects on high fat versus high sugar foods were well described

23   by Koch in 2001. However, only recently were antagonist studies used to facilitate the

24   understanding of the role of CB_1R alone in the selection and consumption of highly

1   fatty or sweet foods. In 2007, a study by Thornton-Jones *et al.* (2007) examined the

2   effects of SR141716A-induced CB_1R blockade on consumption of sweet (10% sucrose)

3   or fatty (10% intralipid) solutions and analysed the lick-based response in a way similar

4   to Higgs *et al*. (2003). Appealingly, this paradigm also compared the hypophagic

5   SR141716A effects with those induced by behavioural manipulations designed to

6   reduce either motivation to feed (pre-feeding) or food palatability (addition of quinine to

7   the intralipid solution and reduction of sucrose concentration to 5%) which, combined

8   with the lick-based microstructure analysis, allowed for more detailed interpretation of

9   the results. Supporting previous findings, this study demonstrated that SR141716A

10 treatment reduced both sucrose and intralipid solution intake, and microstructure

11 analysis revealed that these reductions were due to decreased perceived palatability of

12 ingesta. However, whilst these significant reductions were induced by both 1.0mg/kg

13 and 3.0mg/kg SR141716A for the intralipid solution, only 3.0mg/kg SR141716A

14 significantly reduced sucrose solution consumption. Furthermore, reduced lick duration

15 of intralipid solution observed after drug administration led the authors to suggest that

16 the SR141716A-induced reduction in consumption of this solution was a result of a

17 decrease in motivation to feed, as well as an effect on perceived palatability. In contrast,

18 reductions in sucrose solution intake were less pronounced than those seen for the

19 intralipid solution, suggesting that eCBS may be more strongly implicated in

20 consumption of highly calorific than sweet, palatable food-types. Taken alone, this work

21 suggests that the eCB system can play its role in energy homeostasis via modulation of

22 the motivation to consume fatty foods specifically; this long term effect may be due to

23 changes in meal patterning over the shorter term, particularly with respect to the control

24 of fat intake. Indeed, the work of Thornton-Jones further supports the findings of

1   Arnone *et al.* (1997), Simiand *et al.* (1998) and Koch (2001), as previously discussed,

2   and strengthens the link between CB_1R activation and the control of feeding.

3   Using a different experimental approach, a recent study by Mathes *et al.* (2008)

4   also addressed these issues, and provided further support for eCB system involvement

5   in the specific consumption of highly fatty and/or sweet foods. A 'dessert protocol' was

6   employed, whereby rats were given *ad libitum* access to a wet mash diet made from

7   standard laboratory chow in addition to simultaneous but time limited access to a

8   'dessert' diet high in fat and sugar. Vitally, this methodology gave rats a choice between

9   two different diets, allowing within-group analysis of diet selection. eCB system

10   involvement in the consumption of these two different food-types was assessed by

11   administration of either of the CB_1R antagonists, SR141716A (1.0mg/kg; i.p.) or

12   AM251 (0.3, 1.0 or 3.0mg/kg; i.p.) 30min prior to dessert access. Both compounds

13   significantly reduced caloric intake over a 24 hour period, but this effect was due to a

14   significant decrease in the consumption of the high fat and sugar diet only, with no

15   effect on the consumption of the standard chow. When considered alongside Arnone *et*

16   *al.* (1997), Koch (2001), Simiand *et al.* (1998) and Thornton-Jones *et al.* (2007), these

17   findings suggest that CB_1R-mediation of intake not only modulates intake of fatty food

18   more that sugary food, but does so in a preferential fashion. Unfortunately, although

19   Mathes *et al.*, in the earlier stages of this study, compared the consumption of the high

20   fat and sugar diet with another diet high in sugar only, the effects of CB_1R blockade on

21   this sugar-only diet were not examined.

22   While the use of the previously discussed antagonists has been shown to reduce

23   feeding in a number of models, there is considerable on-going research looking for

24   novel compounds which perform similar actions. Such research is performed to further

1   our understanding of the eCB system and its ligands, whilst also searching for new anti-

2   obesity drugs (for examples please see PSNCBAM-1, Horswill *et al.*, 2007; AM4113,

3   Sink *et al.*, 2007; O-2050, Gardner *et al.*, 2006; MK55-96, Yan *et al.*, 2010; and MK-

4   0364, Fong *et al.*, 2007). While, O-2050 was found to induce significant reductions in

5   motor coordination, which may have been the cause for its effects on feeding

6   behaviours, PSNCBAM-1, AM4113 and MK-5596 have been demonstrated to reduce

7   short term intake and reduce body weight in animal models without apparent side

8   effects. Further work using these novel agents would benefit from the inclusion of meal

9   patterning analysis as this would allow the study of appetitive and/or consummatory

10   behaviours, as well as effects on perceived palatability; such an analysis may shed more

11   light on eCB mediation of feeding behaviours.

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13

1　*Non-Δ⁹THC phytocannabinoid effects on feeding*

2　Very recently it has been suggested that non-Δ^9THC pCBs can also modulate rodent

3　feeding patterns (Farrimond *et al.*, 2010a; Farrimond *et al.*, 2010b) and induce different

4　changes than Δ^9THC, without any apparent non-specific behavioural effects. Before

5　2009 there had been a limited number of studies which demonstrated non-Δ^9THC pCB-

6　mediated feeding effects. Unfortunately these studies were either unrepeated or

7　contradictory. Sofia and Knobloch (1976) reported that the CB_1R agonist CBN reduced

8　intake in rats, an effect yet to be repeated, and that CBD also reduced feeding in rats.

9　However, Wiley *et al.*, (2005) reported that CBD administration had no effect on food

10　intake in mice, a result repeated by Riedel (2009) who observed only a small, non-

11　significant reduction in food intake and weight gain, also in mice. It should be noted

12　that the dose ranges used by Sofia, Knobloch and Wiley are considerably higher than

13　those used by Farrimond and colleagues and, given the well described bi-phasic dose

14　effects of CB_1R activation (Glick *et al.*, 1972) on feeding, this may explain the

15　differences in observed effect patterns. Work in our laboratory has demonstrated that a

16　range of pCBs may have significant effects on feeding patterns. Thirty-six pre-satiated,

17　male, Lister-hooded rats received a purified, *C. sativa*-derived Δ^9THC (hereafter

18　purified Δ^9THC), a synthetic Δ^9THC and a high-Δ^9THC standardised botanical drug

19　substance (BDS), similar in composition to that used in Sativex and containing a typical

20　array of non-Δ^9THC pCBs (high-Δ^9THC BDS; all treatments were dose matched for

21　Δ^9THC content; 0.00, 0.34, 0.67, 1.34 and 2.68mg/kg; p.o.; Farrimond *et al.*, 2010a).

22　Synthetic and purified Δ^9THC administration induced classical Δ^9THC hyperphagia in

23　this study: the latency to the first meal was reduced and both the first hour intake and

24　first meal duration were increased versus vehicle treatments. Intriguingly, the effects of

1   the high-Δ^9THC BDS administered showed significantly reduced hyperphagia in

2   comparison. These results suggested that the combination of pCBs (and, potentially,

3   non-cannabinoid components) in the high-Δ^9THC BDS attenuated the hyperphagic

4   effects of Δ^9THC. Given the constituents of the high-Δ^9THC BDS, (Δ^9THC; 67.0%,

5   Cannabigerol (CBG); 1.7%, Cannabichromene (CBC); 1.6%, CBN; 1.5%, Δ^9THCV;

6   0.9%, Δ^9tetrahydrocannbinolic acid (Δ^9THCA); 0.3%, CBD; 0.3% and Cannabitriol

7   (CBO); 0.2%) it is possible that the pCBs present could have affected feeding

8   synergistically, or that at least one pCB modulated the Δ^9THC-induced hyperphagia.

9   Indeed, the high-Δ^9THC BDS used in Farrimond *et al.,* (2010a) contained known CB$_1$R

10   antagonists (Δ^9THCV, Dennis *et al.*, 2008; Ma *et al.*, 2008; Thomas *et al.*, 2005) in

11   addition to modulators of Ca^{2+} homeostasis (CBD; Ryan *et al.*, 2009) and CBN (Rao *et*

12   *al.*, 2006); which could modulate eCB levels. However, these results do not preclude the

13   possibility that non-cannabinoid material also present in the high-Δ^9THC BDS also

14   contributed to the observed behavioural effects.

15   Consistent with these results we have also demonstrated that non-Δ^9THC pCBs

16   can affect feeding when co-administered with sub-effective concentrations of Δ^9THC

17   (Farrimond *et al.*, 2010b; please also see Riedel *et al.*, 2009 for non-Δ^9THC pCB-

18   induced hypophagia fully discussed below). Ten, pre-satiated, adult, male, Lister-

19   hooded rats received a low-Δ^9THC standardised BDS (low-Δ^9THC BDS; 0.0, 0.5, 1.0,

20   2.0 and 4.0mg/kg; p.o.) containing only 6.9% Δ^9THC, alongside 14.2% other pCBs.

21   Conversely, at these concentrations, rather than having no effect on feeding (as it might

22   be expected, given the use of a prefeed paradigm with an extremely low dose of Δ^9THC

23   and pCBs that have previously been shown to reduce Δ^9THC-mediated hyperphagia),

24   the low-Δ^9THC BDS significantly increased chow intake during the first hour, by

1 significantly reducing the latency to the first contact with food for the highest doses

2 versus vehicle-treatments. These reductions in meal one latency mirror those previously

3 demonstrated for considerably higher concentrations of pure Δ^9THC (Farrimond *et al.*,

4 2010a; Williams *et al.*, 1998). Furthermore, while the latency to the first meal was

5 significantly reduced, the size and duration of this meal was unaffected. Therefore, we

6 suggested that the ratio of non-Δ^9THC pCBs present in the low-Δ^9THC extract

7 significantly increased appetitive (but not consummatory behaviours), since the

8 concentrations of Δ^9THC present were between two- and ten-fold lower than those

9 previously demonstrated to induce hyperphagia (Farrimond *et al.*, 2010b).

10 When these two studies are considered together (Farrimond *et al* 2010a and

11 2010b), a number of suggestions can be made: firstly, since the observed effects of the

12 non-Δ^9THC components of the extracts administered reduced hyperphagia when

13 presented in one ratio and increased hyperphagia when presented in a different ratio, it

14 is highly unlikely that the plant matter also present was responsible for these effects,

15 given that its content did not change between the two tests. Secondly, it is possible that

16 the pCBs present in both studies did not act in isolation such that more than one

17 compound induced the observed effects. Thirdly, these effects could involve non-CBR

18 dependent pathways since several of the non-Δ^9THC pCBs present are known to act via

19 mechanisms only partially connected to CBR (see Izzo *et al.*, (2009) for review).

20 Finally, these results give further credence to the already well supported therapeutic

21 potential of non-Δ^9THC pCBs for the treatment of energy balance disorders. Indeed,

22 non-Δ^9THC pCB-based treatment may prove more tolerable than Δ^9THC treatment

23 alone (e.g. Nabilone) due to the absence of psychotropic side effects associated with

24 non-Δ^9THC pCBs.

1   Whilst a definitive mechanism underlying the findings reported by Farrimond *et*

2   *al.*, (2010a and b) remains unknown, the naturally occurring pCB and CB_1R neutral

3   antagonist Δ^9THCV, which was also present in the extracts employed in Farrimond *et*

4   *al.,* has been considered alone and shown to exert hypophagic actions (Riedel *et al.*,

5   2009). Indeed, given the difficulties associated with the use of the highly specific and

6   potent CB_1R antagonist/inverse agonist SR141716A in the clinic, the use of Δ^9THCV,

7   as a neutral antagonist, to reduce food intake may be of therapeutic value. Riedel *et al.*

8   (2009) demonstrated that purified Δ^9THCV (3.0, 10.0 and 30.0mg/kg; i.p.) significantly

9   reduced chow intake over a period 12 hours and induced weight loss in male C57BL6

10   mice. Subsequent treatment with extremely low Δ^9THCV BDS doses (which contained

11   between 0.1 and 0.3mg/kg Δ^9THC) failed to induce changes to feeding patters; however

12   the Δ^9THC quantities presented were within the range previously observed to affect

13   meal patterning in rats (Farrimond *et al.,* 2010). Unfortunately, meal microstructure was

14   not analysed in this study. In a further experiment, Riedel hypothesised that if CBD

15   (10.0mg/kg; i.p.) were to be co-administered with these very low Δ^9THCV BDS doses,

16   the effects of the present Δ^9THC would be antagonised, and the hypophagic properties

17   previously induced by purified Δ^9THCV would therefore emerge. However, the

18   evidence for *in vivo* antagonism of Δ^9THC by CBD has so far only been demonstrated

19   in a memory task (Fadda *et al.*, 2004), and Riedel observed no significant effects on

20   feeding or weight change following CBD co-administration. The authors suggest that

21   this lack of effect may also be attributed to other pCBs, present in his extract, or simply

22   because the concentrations of Δ^9THCV administered were too low. However, Riedel

23   recorded all feeding data during the light phase of the rats' day during which time little

24   feeding typically occurs. Furthermore, it is also possible that the experimental paradigm

1   used was not optimised for the observation of hypophagia. For instance, introducing

2   food-deprivation prior to dosing could accentuate possible differences between control

3   and conditions measurements, thereby rendering the experimental paradigm more

4   sensitive to Δ^9THCV-mediated reductions in feeding. While, Riedel has confirmed that

5   purified Δ^9THCV can reduce food intake in mice, further experiments which use more

6   sensitive paradigms and analyse meal microstructure after Δ^9THCV BDS administration

7   may prove useful in fully understanding Riedel's data.

8   The work discussed throughout this manuscript demonstrates that some pCBs

9   can alter appetitive and consummatory behaviours separately (e.g. Higgs *et al.*, 2003).

10   As such if it were possible to use at least one non-Δ^9THC pCB to modulate

11   consummatory behaviours (to increase or decrease the quantity consumed during a

12   natural eating event) rather than inducing immediate short term post-administration

13   hunger (the 'munchies', a colloquial term meaning increased hunger normally due to

14   smoking cannabis recreationally) it may be possible to produce a *C. sativa*-based

15   medicine which could control absolute intake quantity. Even though the mechanisms by

16   which the eCB system modulates palatability and therefore diet selection remain

17   unclear, direct effects on diet selection caused by cannabinoid administration have also

18   been demonstrated. Given the presented data which suggests that increased eCB tone

19   leads to increased selection of fatty foods over sweet foods, it is a possibility that non-

20   psychotropic pCBs could also be used to modulate diet selection i.e. to reduce fatty food

21   palatability in obesity. Indeed, putative anti-obesity treatments using pCBs are of great

22   interest. Equally, while some pCBs have been shown to induce significant reductions in

23   food intake and in some cases reduce body weight (e.g. Δ^9THCV), little research is

1   currently considering possible mechanisms by which intake and body weight may be

2   increased, which would be valuable in the treatment of anorectic disorders.

3

4

1   **Conclusions**

2   A considerable quantity of characterisation work has been performed on Δ^9THC-

3   mediated feeding effects, but the specific roles of other pCBs remain less well

4   understood. Work in our laboratory and others suggests that non-Δ^9THC pCBs can have

5   profound effects on feeding behaviours (Farrimond *et al.*, 2010a; Farrimond *et al.*,

6   2010b; Riedel *et al.*, 2009; Sofia *et al.*, 1976; Wiley *et al.*, 2005). However, these are

7   not yet well characterised. As such considerable further work is required to assess the

8   effects of both purified single pCBs as well as combinations of numerous pCBs. With

9   the recent UK approval of the Δ^9THC CBD combination in Sativex, it can be clearly

10   seen that the use of pCBs in the clinic is gaining public acceptance that regulatory

11   bodies are willing to accept plant-derived medicines alongside their synthetic

12   counterparts. Indeed, non-Δ^9THC pCBs may represent an untapped clinical potential for

13   a number of feeding disorders. Such a need is particularly important given the non-

14   specific behavioural side effects associated with the use of Δ^9THC and its analogues in

15   the clinic. Given the encouraging results demonstrating non-Δ^9THC pCB-mediated

16   modulation of feeding, it is tempting to suggest that these compounds may provide at

17   least the basis for novel therapies for the treatment of feeding disorders.

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

2   **Figure 2;** eCB signalling pathways. Upon invasion of an action potential into the

3   synaptic region of the presynapse, the release of neurotransmitter (in this case glutamate

4   or acetylcholine) and the binding of neurotransmitter to postsynaptic receptor sites: the

5   eCBs AEA and 2-AG are produced on demand from arachidonic acid by *N*aPE and

6   DAG respectively and released into the synaptic cleft from the postsynapse (red). Upon

7   neurotransmitter binding to both metabotropic Glutamate and Acetylcholine receptors G

8   subunits disassociate and stimulate an increase in PLC-β levels. This leads to an

9   upregulation of intracellular IP_3 which gates calcium channels on the endoplasmic

10   reticulum thus increasing intracellular Ca^{2+}. Furthermore, the release of $G_{\alpha i/o}$ subunits

11   upregulates the production of cAMP and therefore allows further Ca^{2+} entry into the cell

12   via VGCCs. Equally upon activation of iGluR channels calcium is allowed into the cell.

13   Once released into the extracellular space the endocannabinoids bind to CB_1R sites on

14   the presynapse (green) causing the presynaptic cell to move closer to its resting

15   membrane potential principally by closing VGCCs and opening VGPCs thus reducing

16   the available intracellular Ca^{2+} required to facilitate further neurotransmitter release.

17   **Figure 3;** simplified diagram illustrating retrograde signalling by eCBs in the CNS.

18   Following vesicular neurotransmitter release from the presynaptic cell (green),

19   neurotransmitters bind to receptor proteins (black) on the postsynaptic cell (red)

20   stimulating eCB production and release from the postsynaptic cell. eCBs then diffuse

21   back across the synaptic cleft and bind to CBRs (blue) on the presynaptic cell. This

22   predominately leads to a suppression of $[Ca^+]$ influx but also an increase in $[K^+]$ efflux,

23   thus hyperpolarising the presynaptic cell and inhibiting further neurotransmitter release.

1   **Table 1;** endocannabinergic tone effecter ligands. A brief overview of the CBR ligands

2   discussed throughout this review, giving further background to their discovery and

3   where appropriate their non-feeding effects.

4   Abbreviations: 2-AG, 2-archidonlyglycerol; Δ^8THC, Δ^8tetrahydrocannabinol; Δ^9THC,

5   Δ^9tetrahydrocannabinol; Δ^9THCA, Δ^9tetrahydrocannbinolic acid; Δ^9THCV,

6   Δ^9tetrahydrocannbivarin; AEA, anandamide; CB_1R, cannabinoid type 1 receptor; CB_2R,

7   cannabinoid type 2 receptor; CBR, cannabinoid receptor; CNS, central nervous system;

8   FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; OEA, n-

9   oleoylethanolamide; P-gp, p-glycoprotein; VR_1, transient receptor potential vanniliod 1;

10    $TRPA_1$, transient receptor potential cation channel, subfamily A, member 1.

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