Endocannabinoids in Appetite Control and the Treatment of Obesity

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Abstract: Research into the endocannabinoid 'system' has grown exponentially in recent years, with the discovery of cannabinoid receptors and their endogenous ligands, such as anandamide and 2-arachidonoylglycerol (2-AG). Important advances have been made in our understanding of endocannabinoid transduction mechanisms, their metabolic pathways, and of the biological processes in which they are implicated. A decade of endocannabinoid studies has promoted new insights into neural regulation and mammalian physiology that are as revolutionary as those arising from the discovery of the endogenous opioid peptides in the 1970s. Thus, endocannabinoids have been found to act as retrograde signals: released by postsynaptic neurons, they bind to presynaptic heteroceptors to modulate the release of inhibitory and excitatory neurotransmitters through multiple G-protein-coupled receptor (GPCR)-linked effector mechanisms. The metabolic pathways of anandamide and 2-AG have now been been characterised in great detail, and we can anticipate that these pathways – together with endocannabinoid uptake mechanisms – will complement cannabinoid receptors as targets for the pharmacological analysis of the physiological functions of these substances. Specific insights into the potential role of endocannabinoid-CB1 receptor systems in central appetite control, peripheral metabolism and body weight regulation herald the clinical application of CB1 receptor antagonists in the management of obesity and its associated disorders.

Keywords: Cannabinoid, CB1, CB2, anandamide, 2-AG, rimonabant, surinabant, metabolic syndrome.

CANNABINOID RECEPTORS

Although the use of cannabis (*Cannabis sativa*) for medicinal and other purposes dates back at least four thousand years, understanding of the underlying pharmacology dates back only forty years, and rapid progress has only been made since the discovery of G protein-coupled receptors (GPCR). In the 1960s, Mechoulam determined that cannabis contains more than sixty bioactive 'cannabinoid' compounds [1]. Of these, Δ^9-tetrahydrocannabinol (THC) is widely regarded to be primarily responsible for many of the well-known physiological and psychoactive properties of the plant, and has been the focus for many recent pharmacological and therapeutic developments. It should be noted, however, that relatively little research has been conducted into the actions of the other phytocannabinoids, but ongoing studies suggest that these other compounds (alone, or acting synergistically) may have important and potentially beneficial actions.

Because of their lipophilic/hydrophobic nature, the pharmacological actions of cannabinoids were initially attributed to effects on membrane fluidity, rather than to specific receptor-mediated interactions [2]. However, with the synthesis of high-affinity, stereoselective THC analogues such as CP55940, it became apparent that cannabinoid compounds utilize signal transduction mechanisms akin to those already established for neurotransmitters [3]. Howlett and colleagues reported that THC and its analogues could decrease concentrations of the second messenger cAMP (cyclic adenosine monophosphate) in neuron cultures, and inhibit membrane adenylyl cyclase activity. Moreover, these actions were correlated with the relative potency of cannabinoids in the so-called 'tetrad' mouse behavioural assay (measuring catalepsy, ataxia, antinociception and hypothermia)[4]. Since cAMP formation is regulated by adenylyl cyclase and mediated by G-proteins in response to transmitter-receptor binding, it was deduced that cannabinoid effects involved actions at specific GPCR [5]. The use of $[H^3]$CP55940 enabled the regional distribution and pharmacological characterisation of a specific binding site in mammalian brain for which the ability of cannabinoids to displace $[H^3]$CP55940 correlated with their behavioural activity [5,6].

Two cannabinoid GPCR subtypes have so far been cloned, designated CB1 and CB2. The first (CB1) cannabinoid receptor was discovered by Devane *et al.* in rat brain membranes through analysis of $[H^3]$CP55940 binding characteristics [6]. Subsequently, Matsuda and colleagues in 1990, identified cDNA for an orphan GPCR present in a rat cerebral cortex library [7]. This receptor was unresponsive to a wide variety of neuropeptide ligands, but expression of its mRNA was found to closely parallel the distribution of $[H^3]$CP55940 binding in rat brain. In cells transfected with the new receptor, cannabinoids inhibited adenylyl cyclase, with the potency of this response closely matching the known behavioural potency of the drugs. In the same year Gérard *et al*, cloned a closely homologous orphan GPCR from a human brainstem cDNA library, designated hCB1 [8]. Human and rat CB1 genes encode protein residues of 473 and 472 amino acids respectively, with 97% homology. These proteins share the seven hydrophobic transmembrane domains and residues typical of the GPCR family [7-9]. The mouse CB1 amino acid identity also shows a similar degree of homology to the human (97%) and rat (100%). In fact, the CB1 gene has been identified in a broad range of mammals, with a high degree of conservation across all species. The

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CB1 receptor has also been detected in birds, amphibians and fish [10,11].

At an early stage of research, Shire *et al.* identified an amino terminal splice variant of the hCB1 receptor resulting from an in-frame amino acid deletion [9,12]. Unusually, this form (hCB1a) derives from CB1 mRNA rather than a separate exon. Ryberg *et al.* [13] have recently reported a second variant (hCB1b) with another alteration to the amino terminus, and a distinct distribution pattern within various tissues. These variants are expressed at very low levels, show attenuated affinity for and responses to THC and other synthetic agonists and, unlike the full-length hCB1 receptor, are not activated by physiologically significant levels of any known endogenous agonist. Moreover, similar variants do not occur in rat or mouse (in the absence of the necessary splice consensus sequence), so their significance, if any, to cellular function remains to be determined [14]. However, the possibility remains that these variants may mediate atypical responses to so far unidentified cannabinoid receptor ligands.

Kaminski *et al.* [15] reported a second cannabinoid (CB2) receptor in mouse splenocytes. Subsequently, Munro *et al.* identified the hCB2 cannabinoid GPCR in human HL60 myeloid cells [16], with 44% homology to hCB1 receptor. An orthologue of the human CB2 receptor was subsequently reported in rat spleen, but not in brain. Mouse and rat CB2 share 93% amino acid identity, with the mouse and rat sequences respectively 13 amino acids shorter and 50 amino acids longer than hCB2 [17,18]. More generally, there is much greater inter-species variation in amino acid identity for CB2 than for CB1. The CB2 receptor gene in rat, but not other species, has an intron in the C-terminal region [19]. This disparity may underlie differences in ligand recognition reported between the rat and human CB2 receptor [20].

The expression of cannabinoid receptors in humans has been reported to vary with gender and ethnicity [18], and several hCB1 gene polymorphisms have been identified. A silent mutation resulting in the substitution from G to A at nucleotide position 1359 in codon 453 (Thr) is common in the German population [21]. A simple sequence repeat polymorphism consisting of nine alleles containing (AAT) 12-20 repeat sequences is found in Chinese and Caucasian populations with possible linkage to drug dependency [22]. A 5' CB1 "TAG" haplotype has been reported to display significant allelic frequency differences between drug users and non-users from European-American, African-American and Japanese populations [22]. As we shall discuss later, genomic variation may also play a role in individual susceptibility to disorders of eating and body weight.

Anatomical studies have generally indicated that CB1 is predominantly expressed in the brain and spinal cord [11], and it is thus regarded as the principal target mediating the behavioural and neural actions of cannabinoids. Importantly, the density of CB1 distribution within brain correlates well with the established behavioural actions of THC and other exogenous cannabinoids [7,23-27]. Thus, CB1 is expressed in brain regions that influence mood, motor coordination, autonomic function, memory, sensation, cognition, reward - as well as appetite and metabolism. Expression is particularly abundant in the hippocampus, cerebral cortex, some olfactory regions, caudate, putamen, and nucleus accumbens. In other areas, such as the hypothalamus, CB1 density is relatively low but it has been found that CB1 coupling to G-proteins is more efficient here than in brain regions with higher receptor densities [27,28]. In addition to the CNS, CB1 are also widely expressed in peripheral sites, such as heart, vascular, gut and adipose tissues (see below for further discussion of the latter) [29-37].

By contrast, CB2 was initially reported to be largely confined to peripheral immune and haematopoietic cells [16], and not to be expressed within the CNS. On the basis of this differential distribution of CB1 and CB2 receptors, much of the research relevant to the present review centres on the CB1 receptor. However, recent reports suggest a less clear cut distinction between central and peripheral CB1 and CB2 receptor distribution. Recently, CB2 receptors were detected in cerebellar microglia and neurons and rodent retina, and subsequently in brainstem neurons [38-42]. However, Gong and colleagues have reported that CB2 immunoreactivity is actually present throughout the CNS, being found on neurons and glia [43,44]. The implications of this discovery are likely to require some significant reconsideration of current models of cannabinoid function in neural regulation.

Cannabinoid receptor signal transduction has recently been extensively reviewed by Howlett [45-47]. As members of the class A rhodopsin-like family of GPCR, both CB1 and CB2 receptors act mainly through $G_{i/0}$-type G-proteins [48]. Thus cannabinoids act to inhibit N- and P/Q-type voltage-activated Ca^{2+} channels, open K^+ channels, inhibit adenylyl cyclase, and reduce cAMP formation. There is also evidence that CB1 can act through G_s coupling [49,50], perhaps indicating that there are subtypes of CB1.

A major driving force in recent endocannabinoid research has been the development of selective cannabinoid receptor antagonists (see below) [51-53], together with availability of genetically altered mouse strains lacking CB1 [54,55] or CB2 receptors [56]. Studies using cannabinoid receptor antagonists and receptor knockout mice indicate that some effects of endocannabinoids are mediated neither by CB1 nor CB2 receptors, pointing to the existence of additional as yet unidentified sites of action. Some of these effects may be linked to particular properties of cannabinoid ligands and may be mediated by non-receptor mechanisms, including non-specific interactions at the level of the plasma membrane or intracellular sites. However, there are growing indications of cannabinoid interactions with distinct specific non-CB1/CB2 cannabinoid receptors [57], suggesting that the family of GPCR-type cannabinoid receptors is very likely to be enlarged to incorporate a third subtype.

Recently, two groups have reported cannabinoid binding to the orphan GPCR, GPR55 [58-61]. This receptor, which has so far been identified exclusively in mammalian genomes, has a sequence that is highly divergent to CB1 and CB2 [59], although apparently with conserved sequences in its transmembrane domains [61]. Cannabinoid binding to the receptor reportedly results in activation of RhoA *via* G_{13} G-proteins [60]. GPR55 is expressed within the brain and peripheral tissues, including spleen, gut, and omental adipose tissue. Brown and colleagues have suggested that GPR55 may have acquired affinity for cannabinoids through convergent evolution [59].

ENDOCANNABINOIDS: ENDOGENOUS LIGANDS FOR CANNABINOID RECEPTORS

The existence in mammalian cells of specific membrane receptors for plant-derived substances triggered a search for an endogenous ligand, culminating in the description of several eicosanoid compounds: ethanolamides synthesised from arachidonic acid (for reviews, see [62-65]). In 1992 Devane *et al.* [66] isolated the first endogenous cannabinoid, arachidonoylethanolamide (AEA), subsequently named anandamide after the Sanskrit word for "inner bliss" [66]. Three years later, a second 'endocannabinoid', 2-arachidonoylglycerol (2-AG), was isolated from gut [67] and brain tissue [68]. A third endocannabinoid, 2-arachidonylglyceryl ether (noladin ether) was isolated from porcine brain by Hanus *et al.* [69]. In the ensuing years, other fatty acid ethanolamides with putative endocannabinoid properties have been identified, including *O*-arachidonoylethanolamine (virodhamine) and *N*-arachidonoyldopamine (NADA), but these have been less extensively characterised [70].

These substances are generally agonists at CB1 receptors and have cannabimimetic properties, although some complexities in the actions of the endocannabinoids have been reported. Additionally, there are differences in reports in the relative affinity and efficacy of the endocannabinoids at CB1 and CB2 receptors, depending on the assay, tissue or species being measured [71]. Anandamide, noladin ether and NADA show selectivity for CB1 over CB2, with their relative affinity at CB1 being: anandamide $\geq$ noladin ether $>$ NADA [72]. Anandamide is a full or partial agonist at CB1; while 2-AG is a full agonist in rodent assays, but has been found to lack CB1 activity in human neocortex [71,73]. Adding further complexity, 2-AG has been found to act as an inverse agonist at CB1 with a similar potency to its agonist activity; while anandamide may act as an antagonist at CB2 receptors; noladin ether is a full agonist at CB2 [3,47]. Virodhamine is apparently an agonist at CB2 but has antagonist/inverse agonist activity at the CB1 receptor (*virodha* in Sanskrit means 'opposing') [70]. 2-AG is found at considerably higher levels in brain than anandamide, and it has been suggested that 2-AG is the primary, and optimal, ligand for CB1 (and indeed CB2) receptors [74]. In addition to their actions at cannabinoid receptors, anandamide, 2-AG, virodhamine, noladin ether and NADA have been shown to act as agonists at the vanilloid TRPV1 receptor, indicating ionotropic as well as metabotropic mediation of cannabinoid actions [75,76].

As already noted, there are also a variety of exogenous agonists for cannabinoid receptors, categorised as: classical, non-classical, aminoalkyindoles and eicosanoids (reviewed in [77,78]). Classical agonists consist of dibenzopyran cannabis derivatives (e.g., Δ^9-THC, Δ^8-THC and cannabinol) and their synthetic analogues, such as HU-210, JWH-133, L-759656, L-nantradol, and desacetyl-L-nantradol. Additionally, water-soluble synthetic analogues have been developed, such as O-2694 [79]. Non-classical cannabinoids include bi- and tricyclic analogues of Δ^9-THC that lack a pyran ring, such as CP55940, CP47497, CP55244 and HU-308. The aminoalkyindole group (e.g., WIN55,212-2) are structurally very distinct from the other cannabinoids [80-82]. Finally, the eicosanoid group contains several synthetic anandamide analogues, such as R-(+)-methanandamide (which is resistant to enzymatic breakdown), arachidonoyl-2'-chloroethylamide, arachidonoyl cyclopropylamide, O-689, and O-1812 [70,77,78].

BIOSYNTHESIS, RELEASE AND INACTIVATION OF ENDOCANNABINOIDS

Both anandamide and 2-AG are produced by the enzymatic hydrolysis of precursors derived from the remodelling of membrane phospholipids. Rather than being stored in neurons, AEA and 2-AG are synthesised and released on demand following activation of metabotropic receptors [83, 84], Ca^{2+} influx and activation of Ca^{2+} dependent enzymes [85]. Both are inactivated by intracellular enzymatic hydrolysis [86]. Like other *N*-acylethanolamines, anandamide is synthesised *via* a phospholipid-dependent pathway [87], catalysed by a phospholipase D enzyme (NAPE-PLD) which hydrolyses the corresponding N-acyl-phosphatidylethanolamine (NAPE) [88,89].

A separate synthetic pathway has been determined for 2-AG - the derivation of which was complicated by the fact that this substance is a component of various metabolic pathways, being a precursor and/or degradation product of phospho-, di- and triglycerides, and arachidonic acid [86]. It is therefore important to realise that tissue measures of 2-AG may reflect non-cannabinoid processes. 2-AG synthesis is also distinguished by the fact that it is not solely dependent on neural depolarisation or Ca^{2+} mobilisation. However, the principal 2-AG precursors are *sn*-1-acyl-2-arachidonoylglycerols (DAGs), produced from phospholipid metabolism and remodeling following the stimulation of GPCRs. The conversion of DAGs to 2-AG requires a *sn*-1-selective DAG lipase [90]. Two plasma membrane DAG lipases (DAGα and DAGβ) that are stimulated by Ca^{2+} have been characterised [91]. The synthetic pathways for other putative endocannabinoids remain to be determined. Several non-specific DAG lipase inhibitors have been reported to block anandamide and 2-AG formation. Interestingly, the anti-obesity drug orlistat (tetrahydrolipostatin) has been shown to exhibit relative selectivity for DAGα and DAGβ [63,91].

Anandamide and 2-AG are ultimately degraded by hydrolytic enzymes, expressed by intracellular membranes, to arachidonic acid and ethanolamine or glycerol. One of these enzymes, fatty acid amide hydrolase (FAAH) appears to be primarily responsible for anandamide deactivation, but may also act on 2-AG and virodhamine [63,86,92]. Interestingly, the FAAH gene is upregulated by progesterone and leptin and downregulated by estrogens and glucocorticoids [93,94]. In support of the enzyme's proposed function, FAAH-deficient transgenic mice are more responsive to exogenously administered anandamide, and exhibit 15-fold higher brain levels of anandamide than wild-type mice [95]. Several inhibitors of FAAH are available, although their utility is generally limited by their affinity for cannabinoid receptors, or lack of enzyme-selectivity [96]. One of the more useful compounds for *in vivo* research may be *N*-arachidonoyl-serotonin, which has been shown to increase brain anandamide and 2-AG levels after systemic administration [97]. Development continues however; for example, Muccioli *et al.* [98] recently reported a 5,5'-dipheniyl-tetradecyl-2-thioxo-imidazolin-4-one that inhibits FAAH while being devoid of cannabinoid receptor affinity.

FAAH knockout mice do not have increased brain levels of 2-AG, suggesting that a separate enzyme is responsible for its hydrolysis [99]. The principal candidate for this role is a monoacylglycerol lipase (MAGL), cloned from human, mouse and rat. Like FAAH, MAGL is expressed in brain regions with high CB1 receptor density. And, in accord with the higher concentrations of 2-AG in brain, MAGL appears to be the predominant enzyme [100]. Importantly, FAAH appears to be localized postsynaptically while MAGL occurs in presynaptic neurons [101-104]. If confirmed, these findings have important implications for the respective neuronal roles of anandamide and 2-AG; particularly in relation to the proposed retrograde signalling role of the endocannabinoids. These roles will hopefully be clarified when potent, selective MAGL inhibitors or MAGL-null animals become available [105].

The hydrolysis products of anandamide and 2-AG are immediately recycled into membrane phospholipids, as potential substrates for further endocannabinoid synthesis [86]. However, in addition to hydrolysis, 2-AG may be directly re-esterified into phospholipids [106]. This re-esterification pathway may be key to the inactivation of noladin ether, since the ether bond is resistant to enzymatic hydrolysis [107]. An additional mechanism for endocannabinoid deactivation is oxidation *via* lipoxygenases and cyclooxygenases. It has been reported that the retrograde neural actions of endocannabinoids are potentiated by cyclooxegenase-2 (COX-2) inhibitors, but not by inhibition of FAAH, suggesting a key role for COX-2 in limiting the duration of endocannabinoid action [108]. Oxidation may be particularly important to the inactivation of stable compounds such as noladin and NADA while methylation of the catecholamine moiety of NADA may be catalysed by COMT [109]. Of potential importance is the possibility that stable oxidation products of anandamide and 2-AG could generate compounds such as prostamides and prostaglandin glyceryl esters that might have signalling capacity in their own right [63].

Anandamide and 2-AG are released from cells and reach their targets by cross-membrane diffusion or active transport, facilitated by a so far unidentified membrane protein that has been proposed to facilitate endocannabinoid reuptake [110-113]. Once released, endocannabinoids act primarily on cannabinoid receptors. However, before release, anandamide may also act on intracellular sites on ion channels (possibly on vanilloid TRPV1 receptors and T-type Ca^{2+}channels), to exert a possible autoregulatory, inhibitory influence over endocannabioid actions [75,76].

Endocannabinoids are rapidly cleared from the extra-cellular medium [107,109,114-117]. Given their lipophilic nature, endocannabinoid clearance may occur through simple diffusion, although this would require concomitant reduction of intracellular levels of these molecules, driven by enzymes responsible for their breakdown. Alternatively, it has been suggested that there is an endocannabinoid membrane transporter (EMT). Although the EMT protein/s has not been cloned, and its existence is still questioned [118-119], there are several supporting lines of evidence. Thus: cells that do not express hydrolytic enzymes are able to take up anandamide; the transport process is saturable and exhibits sensitivity to temperature and selectivity for unsaturated long-chain fatty acid amides; anandamide accumulation is evident in synaptosomes from FAAH-null mice, and anandamide uptake inhibitors do not affect FAAH activity [90,110,111,114,120,121].

Although anandamide uptake inhibitors have been developed, they have lacked specificity. For example, the prototypical EMT inhibitor, AM404 [120] can also inhibit FAAH and stimulate TRPV1 receptors [75,122,123]. Recently several compounds have been developed that act as EMT inhibitors with reduced activity against FAAH or TRPV1; such as VDM11 and VDM13 [75]. Newer oleic acid derivatives, such as UCM 707, OMDM-1 and OMDM-2 are more resistant to enzymatic hydrolysis than older, arachidonic acid-derived compounds [124,125]. Clearly, the development of potent and selective inhibitors would be advanced by molecular characterisation of the transporter protein.

Within the brain, CB1 receptors are localised presynaptically on neurons where their activation leads to inhibition of transmitter release [126]. In contrast to other transmitter systems, these presynaptic receptors are classi-fied as heteroceptors, as opposed to autoreceptors, and it is now widely accepted that endocannabinoids are released by neurons to act as retrograde signals - modulating the strength of their synaptic inputs, and contributing to both short- and long-term synaptic plasticity [112,127,128]. Some workers have also provided evidence of postsynaptic CB1 receptors which, if confirmed, would have considerable relevance to endocannabinoid regulation of ion channels [129,130].

CANNABINOID RECEPTOR ANTAGONISTS/INVERSE AGONISTS

The cloning of cannabinoid receptors rapidly led to the development of selective antagonist drugs (for reviews, see [131,132]). The prototypical compounds in this class are the CB1-selective compound rimonabant (SR141716), and the CB2 antagonist SR144528, both diarylpyrazole derivatives developed by Sanofi. It is apparent from the scientific and patent literature that most major pharmaceutical companies have programmes to develop their own antagonist comp-ounds, accelerated as the therapeutic potential of these drugs has become apparent in recent years. These programmes have produced a variety of compounds, such as AM251, AM 281 and LY320135, which have been instrumental in defining the receptor-mediated actions of endocannabinoids and to discern their physiological roles. The principal classes of antagonist include tetrahydrocannabinol and cannabidiol derivatives [133], aminoalkyndoles [134,135], phenyl benzo-furanones [136,137], azetidines [138], aryl-imidazolidine-2,4-diones [139], diarylimidazoles, diaryl-pyrazines, diphen-ylpyridines, diphenyl-phenyls, and diaryl-pirimidine deriva-tives [132].

Rimonabant may be generally considered to act as a competitive antagonist, blocking or reversing the effects of agonists at CB1 receptors, both *in vivo* and *in vitro* (revie-wed in [46,132]). Although rimonabant is CB1 selective, with nanomolar affinity, at micromolar concentrations it can also interact with CB2 receptors [140-142]. At higher concentrations, the drug may be a TRPV1 receptor partial agonist [143,144], as well as an antagonist at adenosine A_1 receptors [145]. Rimonabant has also been reported to block

potassium and L-type calcium channels [146,147]. However, a much discussed feature of rimonabant and other CB1 antagonists is their apparent inverse agonist activity (for a review, see [148]). Thus, these drugs can produce effects in some assays that are the opposite of those induced by agonists, and this may indicate the possibility of constitutive (ligand-independent) activity of CB1 receptors. A recent suggestion is that rimonabant-like drugs may act as neutral antagonists to displace agonists from one site on the CB1 receptor, but exert inverse agonist effects by acting at a separate allosteric site, so modulating the constitutive activity of the receptor [148]. There is some debate about whether constitutive activity occurs *in vivo*, and whether the ability of an antagonist to suppress a behaviour normally stimulated by an agonist may not be more parsimoniously explained by simple antagonism of tonic endocannabinoid activity [105,145]. It is likely that rimonabant and its analogues are neutral antagonists at low concentrations and inverse agonists at high concentrations [3,148]. Nevertheless, a number of laboratories are pursuing the development of neutral antagonists with no inverse agonist profile, with several promising candidates such as NESS 0327 [140] and O-1184 [149], and the phytocannabinoid tetrahydrocannabivarin (THCV) [150].

The focus of the remainder of this review will be the application of CB1 receptor antagonists in relation to overconsumption and obesity. Rimonabant (Acomplia) has recently completed several Phase III trials in Europe and North America. The therapeutic targets proposed for the drug encompass obesity, metabolic syndrome, diabetes and cardiovascular disease. More recently a close analogue of rimonabant (SR147778, surinabant) has begun trials for similar applications. Underlying these developments are the established potency of these compounds to suppress food intake and body weight. These issues will be discussed in more depth below. However, it is clear that a principal reason for initially examining the effect of CB1 antagonists on eating was the long-established ability of cannabis and its extracts to promote overconsumption. It is sensible therefore to provide some background to this action of cannabis, in order to explore the significance, and mode of action, of endocannabinoid systems in the physiological and behavioural regulation of appetite and body weight.

ENDOCANNABINOID IN THE REGULATION OF APPETITE, METABOLISM AND BODY WEIGHT

The appetite stimulating action of cannabis and its extracts has been well-documented for many centuries [151]. It is now reasonable to assume that cannabis hyperphagia is largely attributable to THC actions at brain CB1 cannabinoid receptors, and reflects a biologically significant role of endocannabinoids in appetite processes. However, despite recent advances in cannabinoid pharmacology and the aforementioned promotion of CB1 antagonists in an anti-obesity role, the past decade has seen surprisingly little progress in our understanding of the mechanisms whereby exogenous or endogenous cannabinoids promote eating. Remarkably, there have been only a handful of papers actually assessing the actions of the endocannabinoids themselves in this area. Hypotheses about the likely behavioural role of endocannabinoids are unfortunately too dependent on presumptions derived from a restricted number of studies with THC in people, and on anecdotal reports or subjective accounts of cannabis users. Moreover, as we shall see, antagonist-driven research is moving the emphasis from analysis of endocannabinoid involvement in central appetite processes to peripheral metabolic factors. While this re-emphasis has been accelerated by recent discoveries of CB1 expression in gastrointestinal, adipose, pancreatic and hepatic tissues [152-154], it is necessary to emphasize that peripheral endocannabinoid processes related to energy storage and metabolism may also affect appetite. Therefore, a fuller understanding of how endocannabinoids translate energy requirements into the expression of appetite and eating behaviour are still essential.

The limited literature in relation to cannabinoids and human appetite has been reviewed extensively elsewhere [151,155,156]. The general finding in healthy volunteers (frequently experienced marijuana users) are substantial increases in caloric intake, most frequently derived from snack foods [157-160]. These effects are apparent after acute and chronic dosing (typically in the form of cannabis cigarettes, and less frequently oral THC administration). The usual assumption is that THC predominantly enhances the orosensory qualities of ingesta, and particularly sweet, palatable foods. Such an action is reinforced by an oft-cited study by Abel [161], in which smoking cannabis induced dramatic overconsumption of marshmallows (albeit that was the only food available). One other study examined acute oral THC effects on the consumption of chocolate milk shakes, where in addition to elevated hunger ratings, participants reported enhanced ‘food appreciation’ [162]. However, we have recently obtained data indicating that THC can have broad, dose-related, effects on appetite that are not restricted to specific flavours or food types (Townson and Kirkham, unpublished observations). It is probable, but untested, that these actions are CB1 receptor-mediated, since the broader psychological actions of cannabis in people are reversed by the selective CB1 antagonist rimonabant [163]. Additionally, a small number of clinical trials have assessed the possible benefits of cannabinoids in the treatment of wasting and appetite loss in cancer cachexia and AIDS. Treatment with THC (as the synthetic form, dronabinol) improved appetite ratings, increased food intake, and attenuated weight loss or induced weight gain [164-167]. Clearly, this is a very narrow research base on which to build any detailed model, and the psychological, behavioural and metabolic actions of THC require considerably more detailed investigation.

The animal literature on THC is not much more extensive, and contains as many reports of hypophagia as of hyperphagia – resulting from the use of very high, sedative doses that are incompatible with feeding. Nevertheless, THC has been shown to stimulate feeding in a variety of animal models, after systemic or central administration [168-170]. Importantly, THC hyperphagia in animals has been shown to be mediated by CB1 receptors, being reversed by rimonabant, but not the CB2 selective antagonist SR144258 [171]. The hyperphagic effect of THC in rats is also remarkably potent, causing animals to overconsume even when replete. Interestingly, low doses of Δ^8-THC, have been reported to have significantly greater hyperphagic potency than Δ^9-THC, with fewer cannabimimetic side effects [172].

Such data indicate the importance of exploring the behavioural actions of other phytocannabinoids.

The hyperphagic actions of THC have lately been replicated with the endocannabinoids anandamide, 2-AG, and the putative endogenous CB1 agonist, noladin ether. These substances increase food intake in rodents following systemic or central injection, and their actions are blocked by rimonabant but not SR144528 [32,173-177]. Importantly, anandamide and 2-AG will promote feeding when administered into brain regions associated with eating motivation, including hypothalamic nuclei and the shell of the nucleus accumbens [155,175,176]. These data therefore strongly support a key role for endocannabinoids in the control of eating.

ANORECTIC ACTIONS OF CANNABINOID RECEPTOR ANTAGONISTS

Complementing the actions of CB1 agonists, is the already intimated ability of CB1 receptor blockade to suppress eating in laboratory species. Indeed, a role for endocannabinoids in feeding was proposed on the basis of the initial demonstrations of the anorectic action of rimonabant [178,179]; potentially indicating tonic endocannabinoid activity in feeding-related systems. Reliable anorectic actions of other CB1 antagonists (e.g. AM281, AM251, O-3259 and O-3257) have since been reported. Intake suppression has been observed following systemic or central administration in satiated or food-deprived animals, and after acute or chronic treatments [180-185]. Sanofi-Aventis has recently reported the ability of a new CB1 antagonist, SR147778 (surinabant), to dose-dependently reduce food intake in fasted or non-deprived rats, and to reduce sucrose consumption in rats and mice [186]. In the most recently published study, Plummer *et al.* [187] provide a preliminary report of the anorectic actions of two novel 4,5-diarylimidazoles on *ad libitum* feeding in dietary obese rats. These CB1 inverse agonists were found to dose-dependently suppress food intake and weight gain after acute treatment, with intake suppression evident over 18 h of testing. A number of studies have also investigated the effects of CB1 blockade in genetic models of obesity. Thus rimonabant and AM251 have been shown to exert reliable effects on food intake and body weight in obese *fa/fa* rats, and *ob/ob* and agouti yellow *A(y)* mice, often with greater potency than in lean littermates [188,189].

The ability of CB1 antagonists to suppress food intake soon led to assessment of their potential as anti-obesity treatments. Consequently, there has been considerable interest in the chronic effects of these drugs on intake and body weight. In an early study, Colombo and colleagues [180] demonstrated that daily administration of rimonabant suppressed intake of lab chow and induced persistent weight loss in rats. Although tolerance to the drug's anorectic action was apparent after 5 days, suppression of body-weight gain was evident across the full course of a 14-day experiment. More recently, Vickers *et al.* [188] demonstrated that sub-chronic, oral treatment with rimonabant dose-dependently decreased food (chow) intake and body weight gain in both lean and genetically obese Zucker (*fa/fa*) rats. Once again, the intake-reducing effects waned after 4 days in lean animals, but reduction of body weight gain was maintained over 28 days of treatment. These effects were greater in obese Zucker rats than in lean controls, with daily food intake initially reduced by as much as 40% and persistent weight loss evident over the first 2 weeks of treatment. Moreover, the reduction in potency of the drug's anorectic action was considerably delayed in obese animals, although their intake subsequently remained lower than in vehicle-treated controls. Even after the anorectic action diminished, the rate of weight gain remained significantly suppressed in antagonist-treated rats. Withdrawal of rimonabant on day 28 resulted in rebound hyperphagia and significant weight gain [188]. The mechanisms underlying this differential tolerance to rimonabant's anorectic action remains to be explained; however rapid tolerance to other effects of the drug have also been reported [190,191].

The effects of chronic CB1 blockade has also been addressed in mice made obese through the provision of a high fat diet. This diet-induced obesity is a good model for the commonest form of human obesity and its consequences, including visceral obesity and type 2 diabetes. Ravinet-Trillou *et al.* [192] reported that daily rimonabant administration reduced food intake by almost 50% during the first week of a 5-week study. This initial anorectic effect gradually diminished, but intake remained suppressed compared to vehicle-treated controls throughout the whole test period. Overall, body weights were reduced substantially after 1 week and stabilized at that lower level until the end of the experiment. Carcass analysis showed that rimonabant significantly reduced adipose stores, halving the proportion of body fat seen in controls fed the same high fat diet, while preserving lean mass. A follow-up study confirmed these effects and demonstrated that the drug-induced changes in intake and body composition were dose-dependent. Additionally, elevated plasma levels of insulin and leptin, and insulin resistance that accompany the development of obesity were substantially reduced by antagonist treatment. Similar effects of rimonabant have been reported by Poirier *et al.* [193], who also demonstrated that the drug-induced weight loss and improved glycaemic control closely matched the consequences of shifting untreated dietary obese mice from a high fat to a low fat diet.

Hildebrandt and colleagues [194] confirmed the general effectiveness of chronic CB1 receptor blockade in dietary obesity using the rimonabant analogue, AM251. The drug dose-dependently suppressed intake, initially by as much as 60% below control levels. Significant, dose-related weight loss was evident after three days of treatment and was maintained over an initial 2-week period of daily oral administration; with significant intake suppression evident until day 12. Rebound hyperphagia was apparent during a subsequent drug-free inter-treatment phase, but body weight gain was negligible until after 4-5 drug-free days. Reliable anorexia and weight loss were reinstated when AM251 treatment was given for a further 2 weeks – again with very marked initial intake suppression. In this second drug stage, tolerance to the intake suppressing actions of the drug were evident somewhat earlier, but weight loss was maintained with marked reductions in adiposity. Plasma leptin and cholesterol levels were also significantly reduced at the highest dose, with an additional tendency for plasma insulin to be reduced [194]. The ability of AM251 to attenuate eating and accelerate weight loss with an interrupted dosing

regime suggests that tolerance to the anorectic effects of CB1 antagonists may not be an obstacle to the long term application of these drugs in the treatment of obesity.

It is notable that one group has reported that a single dose of AM251 can lead to intake suppression for up to 6 days post-treatment [195], despite the reported half-life in mouse brain for this compound being of the order of several hours [196]. McLaughlin *et al*. [197] have also reported that the motivational effects of rimonabant and AM251 have a long duration, with significant suppression of food-motivated behaviour evident for up to 22 hours after administration. Although such a persistent effect has to be clearly separated from non-specific actions (such as the induction of conditioned taste aversion; see below), the advantages to therapeutic regimes of intermittent, long-lasting antagonist treatments and the avoidance of tolerance are obvious.

CB1 RECEPTOR KNOCKOUT MICE

The availability of CB1 receptor knockout mice ($CB1^{-/-}$) has provided important supporting evidence for endocannabinoid involvement in appetite regulation. Ravinet-Trillou [192] characterized the phenotype of these animals when fed either standard chow or a high fat diet. When maintained on chow, $CB1^{-/-}$ mice were leaner and slightly hypophagic compared to the wild-type ($CB1^{+/+}$) animals. When fed the palatable, high-fat diet, $CB1^{-/-}$ mice did not display the hyperphagia characteristic of the wild-type and did not develop obesity. Additionally, although $CB1^{-/-}$ mice did display a preference for the high-fat diet, this was acquired more slowly than in $CB1^{+/+}$ littermates [192]. Knockout mice also show reduced consumption of sucrose than the wild type [198]. Additionally, Di Marzo *et al*. [199] have shown that $CB1^{-/-}$ animals display a reduced hyperphagic response to fasting, eating less than wild type littermates after 24-h food deprivation.

SPECIFICITY OF ANTAGONIST EFFECTS ON FOOD INTAKE

As already noted, rimonabant and its analogues have inverse agonist properties in some assays, suggesting that suppression of food intake may reflect this 'inverse cannabimimetic' action rather than competitive blockade of endocannabinoid tone [148]. Rimonabant may be a neutral antagonist at low doses and an inverse agonist at higher concentrations, so the fact that relatively high doses of these drugs (>3 mg/kg) are required to inhibit feeding may be significant. However, low rimonabant doses (<1 mg/kg) effectively attenuate the feeding actions of exogenously administered endocannabinoids, which would support the active role of CB1 agonists in the stimulation of feeding. An additional concern may be that at high doses rimonabant can bind to CB2 receptors [3]. However, the inability of the selective CB2 antagonist SR144528 to block cannabinoid-induced feeding [171], or to suppress feeding in its own right [185] supports the primary involvement of CB1 receptors in rimonabant-anorexia.

Another important issue in relation to the mechanisms whereby antagonists suppress food intake relates to the possibility of induction of non-specific malaise, or of behaviours that are incompatible with the expression of eating. CB1 antagonists have been reported to have anxiogenic activity, and to promote behaviours such as wet dog and head shakes, forepaw fluttering and facial rubbing that might interfere with feeding [190,200]. The antagonists may also have effects on general activity (for example, AM251 has been shown to dose-dependently increase wheel running in mice) that might contribute to reduced food intake and weight loss [189]. It should be noted that non-specific effects are not universally reported at lower anorectic doses. However, there is some evidence for the possibility that antagonists may induce conditioned taste aversion (CTA), and that this effect may partially account for intake suppression. For example, McLaughlin *et al*. [197] reported that AM251 dose-dependently reduced consumption of a novel, flavoured solution with which it had been paired 4 days previously. Additionally, the antagonist induced aversive gaping responses to saccharine in taste reactivity tests. Such effects are clearly of concern in trying to interpret the anorectic actions of CB1 antagonists (and particularly in relation to their possible application in human obesity). Further research into the extent to which side effects of CB1 antagonists may involve modulation of serotonergic, dopaminergic or cholinergic circuits is also indicated by recent research [201-203]. Illness-inducing effects of the antagonists may be independent of any specific appetite suppression, and instead involve CB1 receptors expressed in the brain stem dorsal vagal complex associated with triggering emetic responses [204,205]. Indeed, rimonabant has been shown to induce emesis at higher systemic doses [206]. By contrast, exogenous cannabinoids and anandamide will suppress vomiting and conditioned rejection responses to flavours associated with illness [207,208] (conversely, 2-AG has been shown to have emetic actions [206]).

ENDOCANNABINOIDS AND APPETITE: BEHAVIOURAL MECHANISMS

Hypotheses about how cannabinoids affect eating motivation generally focus on the increased sensitivity to the sensory properties of foods and apparently preferential effects on preferred, highly palatable foods, noted above. Rimonabant's ability to suppress ingestion was initially reported to be enhanced when animals were fed sweet, palatable foods. Additionally, intake suppression was not restricted to food, as sucrose and ethanol drinking were also reliably affected (although water drinking in thirsty animals was unaffected). These combined effects lead to the notion that the drug modifies the appetitive value of ingesta *via* actions on reward pathways [178,179].

Current research supports a role for endocannabinoids in both incentive and reward processes that control the appetitive and consummatory aspects of eating motivation [209,210]. Rats will work harder to obtain palatable ingesta after administration of CB1 receptor agonists, while antagonist treatments attenuate responding [211,212]. Additionally, $CB1^{-/-}$ mice show lower levels of responding for sucrose in operant situations than wild type mice [213]. It should be noted that antagonist effects on operant responding are also evident with bland foods [214,215], and rimonabant is equi-anorectic when tested with foods of differing macronutrient content [216]. This suggests that endocannabinoids modulate appetitive processes *per se*, to provide a general gain in the incentive value of food. Detailed observational analyses and meal pattern analysis

reveals that stimulation of CB1 receptors directly increases the salience of food, irrespective of need or energetic status. Thus, exogenous and endogenous cannabinoids can induce feeding almost as soon as food becomes available; increased food intake after cannabinoid treatment primarily involves the advance of meal onset, rather than a marked increase in meal duration or meal size. Crucially, once initiated, the subsequent pattern of cannabinoid-induced feeding behaviour is identical to that of untreated rats feeding spontaneously under home cage conditions [151,176,217]. This indicates that cannabinoids provoke feeding through adjustments to natural feeding processes rather than induction of stereotyped behaviours [185]. Overall, the ability of agonists to increase responding for food and to specifically reduce eating latency implies that stimulation of CB1 receptors directly activates eating motivation.

Of the various brain loci linked to feeding, the shell subregion of the accumbens (AcbSh) has particularly strong associations with appetitive processes [218]. The AcbSh contains a relatively high density of CB1 receptors [23,27] and is particularly sensitive to endocannabinoid-induced feeding. Administration of 2-AG or anandamide into this region induces substantial short-term, rimonabant-reversible hyperphagia [176]. In line with the notion that cannabinoids increase the incentive value of food, intra-accumbens 2-AG also significantly advances the onset of feeding without markedly affecting other meal parameters. Moreover, the rapidity of onset and magnitude of 2-AG hyperphagia injected into the AcbSh are greater than the effects of anandamide or noladin ether seen after peripheral administration or injection into other brain sites [173,175,177]. Importantly, acute food deprivation significantly increases anandamide and 2-AG levels within the forebrain regions containing the AcbSh [176]. These changes are likely to underlie the significant enhancement of rimonabant anorexia in food-deprived rats compared to non-deprived animals [151,154]).

Mesolimbic dopamine neurons, arising in the ventral tegmental area (VTA) and projecting to the nucleus accumbens, are central to incentive processes in feeding [219,220]. Food stimuli cause dopamine release in the nucleus accumbens after deprivation, or if the food is novel or palatable. There is growing support for complex interplay between endocannabinoids and dopamine on the activity of these mesolimbic circuits [221-223]. Thus, doses of THC within the hyperphagic range stimulate dopamine release in the nucleus accumbens [224,225], and Verty *et al.* [226] have reported that THC hyperphagia is attenuated by a behaviourally silent dose of the D1 antagonist SCH23390. Additionally, SCH23390 has been shown to increase anandamide levels in limbic forebrain, while the D2 antagonist reticlopride elevates 2-AG levels [2]. While much more research is required to unravel these interactions, there is growing support for co-operation between endocannabinoids and dopamine in the orientation of an animal towards motivationally significant stimuli (like food), and the elicitation of appropriate behavioural responses (such as food seeking) [221-223,227].

The hypothalamus also plays a key role in integrating the multiple chemical and behavioural components of feeding and weight regulation, and is functionally linked to the accumbens [228,229]. It is therefore significant that not only will cannabinoid administration into hypothalamic nuclei induce eating, but that hypothalamic endocannabinoid activity apparently changes with nutritional status and the expression of eating behaviour. For example, levels of 2-AG (but not anandamide) are increased in the hypothalamus after 24 hour food deprivation in rats [176] and mice [230]. Interestingly, 2-AG levels decline as animals eat, falling to control levels as animals satiate [176]. These changes are consistent with the behavioural actions of cannabinoids and support endocannabinoid involvement in eliciting eating motivation [155,217]. Additionally, CB1 receptors located in feeding-relevant hindbrain areas, such as the dorsal motor nucleus of the vagus (DMV) and the nucleus tractus solitarius (NTS), may also be subject to cannabinoid regulation [231]. Thus, the cannabinoid receptor agonist CP55,940 has greater hyperphagic potency when administered into the fourth ventricle than when injected into the third ventricle [232].

In addition to appetitive processes, endocannabinoids may also mediate the palatability, or pleasure response to foods. Such a role is evident in anecdotal reports of cannabis users [233], and animal studies show that CB1 stimulation or blockade/deletion can respectively render food more or less pleasurable. CB1 receptor blockade was reported to preferentially attenuate the intake of palatable, sweet foods [178,179] and reduce operant responding for sweet food [215]; while CB1 knockout mice consume less sucrose than wild types [198]. The actions of exogenous and endogenous CB1 agonists on the microstructure of sucrose drinking match those observed in drug-free animals when the palatability of the test solution is increased [234]. Conversely, rimonabant alters drinking in a way that is consistent with a reduction in palatability. Additionally, $CB1^{-/-}$ mice are less responsive to sweet taste, consistently drinking less of a range of sucrose solutions than the wild type [213]. As already noted, key components of the neural mechanisms underlying food palatability lie within the AcbSh and 2-AG administered into this site produces a profound hyperphagic response [176]. In taste reactivity tests, intra-accumbens administration of anandamide specifically increases the number of positive ingestive responses to intra-oral infusions of sweet solutions, indicating that anandamide specifically enhances their hedonic impact [235]. Moreover, accumbens CB1 receptors are downregulated in rats that overconsume palatable foods [236], presumably due to increased activation of these receptors by endocannabinoids.

Endocannabinoids may have important functional relationships with the endogenous opioid systems that have an established role in mediating food palatability [237-239]. In rats, the hyperphagic action of THC is reversed by the general opioid receptor antagonist, naloxone [171]. Importantly, the facilitatory effects of a CB1 agonist on responding for palatable solutions were reversed by both a CB1 antagonist and naloxone [211]. Moreover, low doses of rimonabant and opioid antagonists that are behaviourally inactive when administered singly, combine synergistically to produce a profound anorectic action when co-administered [183,240,241]. Intra-accumbens administration of either morphine or anandamide increases the liking of sweet solutions [235,242], and there is ultrastructural evidence that

cannabinoid-opioid interactions are mediated by activation of CB1 and μ-opioid receptors within the same, or synaptically linked, reward-relevant neurons in the AcbSh [243]. Moreover, systemic administration of THC has been shown to stimulate β-endorphin release in both the VTA and AcbSh [244], and CB1 agonist-induced DA release in the accumbens is blocked by intra-VTA infusion of the μ1-selective antagonist, naloxonazine [245]. Solinas and Goldberg [246] have reported that THC and morphine dose-dependently increased break points for food reinforcement, while rimonabant and naloxone dose-dependently decreased break points. Furthermore, THC's effects were blocked by naloxone, and morphine's effects were blocked by rimonabant. These data suggest that activation of endocannabinoid and endogenous opioid systems jointly facilitate the motivational effects of food.

CENTRAL ENDOCANNABINOIDS AND THE OREXIGENIC AND ANOREXIGENIC PEPTIDES

There is a growing body of evidence to link endocannabinoids with a wide range of other factors implicated in the regulation of appetite [152,247,248]. Noteworthy are apparent interactions between endocannabinoids and the adipokine leptin which is considered to be important to the regulation of long term food intake and weight control [249-251]. Di Marzo *et al.* [199] reported that exogenous leptin administration, which exerts an anorectic action, suppresses hypothalamic endocannabinoid levels in normal rats; while genetically obese, chronically hyperphagic rats (*fa/fa*) and mice (*ob/ob*) express elevated, leptin-reversible, hypothalamic anandamide or 2-AG levels. Rimonabant has been shown to be more effective in suppressing food intake in obese Zucker (*fa/fa*) rats [188], which may indicate a greater sensitivity to the drug as a consequence of the upregulation of central cannabinoids. Agouti yellow (A^y) mice are reportedly leptin-resistant, raising the possibility that upregulation of endocannabinoids underlies the hyperphagia and obesity in these animals [189]. A relationship between leptin and endocannabinoids is also suggested by the finding that $CB1^{-/-}$ mice have an enhanced sensitivity to the intake suppressing actions of leptin [252]. However, there are contradictory findings: for example, Harrold *et al.* [236] found that although dietary-obese rats were hyperleptinemic, there was no correlation between plasma leptin levels and hypothalamic CB1 expression.

In their original report of rimonabant's anorectic action, Arnone *et al.* [178] also reported that the drug could block the ability of neuropeptide Y (NPY) to increase intake of a palatable sucrose solution. Poncelet *et al.* [198] reported that rimonabant can also prevent NPY-induced eating. NPY-hyperphagia is abolished in $CB1^{-/-}$ mice, although rimonabant is as effective in reducing food intake in NPY knockout mice as in wild type [199]. There also additional evidence for cannabinoid interactions with the hypothalamic melanocortin system. Verty *et al.* [253] reported that in rats sub-anorectic, intracerebroventricular (icv) doses of the melanocortin MCR4 receptor agonist α-melanocyte stimulating hormone (α-MSH) and rimonabant combined synergistically to suppress feeding. Feeding stimulated by the MCR4 antagonist JKC-363 was dose-dependently attenuated by rimonabant, whereas THC-induced eating was unaffected by α-MSH. These results were interpreted as indicating that CB1 receptors are located downstream from melanocortin receptors, and that endocannabinoids exert an inhibitory action on the ability of the melanocortin system to inhibit feeding. Additionally, obese agouti A^y mice with defective hypothalamic melanocortin-4 receptor (MCR4) signaling are sensitive to the appetite and weight suppressing actions of AM251 [189].

Cota and colleagues [152] have shown co-localization in the paraventricular hypothalamus (PVN) of the CB1 receptor with the anorexigens cocaine amphetamine-regulated transcript (CART), corticotropin-releasing hormone (CRH) and orexigenic melanin-concentrating hormone (MCH). There is also evidence for functional interactions between endocannabinoids and the orexigenic peptide, orexin A, with possible cross-talk between CB1 receptors and the orexin OX1R receptor [254]. Additionally, $CB1^{-/-}$ knockout mice show higher levels of mRNA for the anorexigen CRH [255], and CRH1 receptors are co-localized with CB1 in several brain regions, including the lateral hypothalamus [256].

Recently, evidence has been obtained for interactions between brain endocannabinoids and the orexigenic gut-brain peptide, and putative hunger signal, ghrelin. Ghrelin is released principally from gastric tissues, but also synthesised within the hypothalamus [257-260], and circulating ghrelin levels rise in advance of meals and decline rapidly post-prandially. Feeding stimulated by intrahypothalamic (PVN) ghrelin injection is blocked by pre-treatment with rimonabant, suggesting that expression of ghrelin hyperphagia is dependent on an intact central endocannabinoid system [261].

PERIPHERAL INFLUENCES ON ENDOCANNABINOID APPETITE CONTROL

In addition to the central nervous system, cannabinoids and CB1 receptors are also present in gastro-intestinal, adipose, pancreatic and hepatic tissues, and play a role in regulating gut motility and gastrointestinal enzyme secretion [153,262,263]. Additionally, there are data suggesting the influence of peripheral endocannabinoids on the regulation of appetite and body weight [152,154,264]. A role for peripheral endocannabinoids in the control of feeding was proposed after observations that anandamide is synthesised within gut tissues, with small intestine concentrations increasing in 24-hour fasted rats [32]. Moreover, the respective hyperphagic or anorectic actions of intraperitoneal anandamide and rimonabant were attenuated by capsaicin deafferentiation of peripheral sensory nerves. These findings were interpreted as indicating a possible role for intestinal anandamide as a "hunger signal". This hypothesis was based on the finding that anandamide hyperphagia and rimonabant anorexia could only be obtained after systemic, but not central, administration. However, central administration of anandamide, 2-AG, THC, CP55940, and CB1 antagonists *has* been demonstrated to exert reliable effects on intake [175,176,181,232,237], so an exclusively peripheral origin for an endocannabinoid appetite stimulus appears to be untenable.

However, there is other evidence for peripheral cannabinoid contributions to appetite stimulation. For example, support for functional peripheral interactions between endocannabinoids and ghrelin comes from a report by Cani and

colleagues [265]. They found that systemic rimonabant treatment markedly suppressed fasting-induced increases in plasma ghrelin; an effect associated with an almost complete suppression of eating in the 2 h after food was restored. Given the location of both CB1 receptors and ghrelin synthesis within the gastric fundus, this finding may indicate local endocannabinoid regulation of ghrelin release and subsequent mediation of the circulating ghrelin signal *via* arcuate nucleus neurons [266]. A possible additional route by which peripheral cannabinoids might affect central behaviour regulation is suggested by the finding that gastric and duodenal vagal afferents express CB1 receptors [267]. Moreover, vagal CB1 expression was increased by prolonged food deprivation and rapidly reduced on re-feeding. These same afferents express receptors (CCK1) for the putative satiety hormone cholecystokinin (CCK). Interestingly, the respective effects of fasting and re-feeding on CB1 expression were blocked by the CCK1 receptor antagonist, lorglumide, and mimicked by CCK injection in fasted animals. It was concluded that CCK may naturally inhibit the ability of peripheral anandamide to stimulate feeding *via* vagal activity, and so fasting may release vagal cannabinoid signals from CCK inhibition [267]. More recently, it has also been reported that the reduction of vagal CB1 expression seen in fasted rats that were re-fed is inhibited by ghrelin administration, indicating further complexity to the influence of cannabinoid systems on orexigenic and anorexigenic signals [268].

ENDOCANNABINOID REGULATION OF FAT METABOLISM

Recent studies have indicated important cannabinoid influences on energy regulation that are distinct from their direct actions on appetite, and which may involve the co-ordination of both central and peripheral CB1-mediated processes [152]. Thus, CB1 antagonists have been shown to reduce adiposity in diet-induced obese mice and genetically obese rodents, independently of their primary anorectic actions [188,192,193]. As already noted, chronic CB1 blockade only transiently suppresses food intake, while weight loss persists for the duration of treatment. Moreover, it is argued that weight loss in rimonabant-treated animals exceeds that which could result from reduced food intake alone. Thus, pair-feeding tests (in which control animals receive the same amount of food voluntarily consumed by antagonist-treated animals), showed that weight loss was greater with rimonabant than in pair-fed controls. Additionally, when deprived of food for 24 hours, rimonabant-treated obese mice lose more weight than similarly deprived controls [192].

CB1 knockout mice are resistant to diet-induced obesity, and do not exhibit the insulin resistance normally occurring in high fat-fed mice [252]. Additionally, in obese Zucker rats and dietary obese mice, rimonabant lowers plasma free fatty acid levels, corrects hyperglycaemia, reduces plasma insulin levels, counters insulin resistance, reduces serum triglycerides, and can restore normal high-density:low-density lipoprotein cholesterol ratios (HDLc:LDLc) through a reduction of low LDLc; plasma leptin levels are also substantially reduced [192,193]. Notably, in dietary obese mice maintained on a high fat diet, these changes persist long after the transient anorectic actions of rimonabant [193]. Antagonist treatments may therefore interfere directly with cannabinoid processes that regulate fat deposition in adipose tissues, fatty acid oxidation or glucose homeostasis. It is therefore of great significance that CB1 receptors are expressed by adipocytes in normal animals, but not CB1 receptor-deficient mice, and that these receptors are upregulated in obese *fa/fa* rat adipocytes [269]. Stimulation of these receptors can induce lipogenesis; potentially by promoting free fatty acid formation, since rimonabant can reduce lipoprotein lipase levels *in vitro* [152]. Interestingly, exercise (voluntary wheel-running) has been shown to augment the actions of AM251 on weight loss in lean and obese agouti A^y mice [189]. Thus, lower doses of the drug were required to reduce food intake and body weight when combined with exercise. The authors proposed that CB1 blockade and exercise may have complementary effects on sympathetic activity, which in turn stimulates lipolysis.

Roche and colleagues have recently confirmed that cannabinoid receptors are expressed in human adipocytes (and pre-adipocytes), in visceral (omental) and subcutaneous adipose tissue from males and females [270]. They demonstrated the presence of functional CB1 receptors, which respond to 2-AG stimulation with increased intracytoplasmic cAMP levels – an effect which was reversible using the selective CB1 antagonist AM251. This group also reported the expression of functional CB2 receptors in the same tissues.

The recent discovery that hepatocytes express CB1 receptors provides further evidence of crucial endocannabinoid influences on lipogenesis [154]. Thus, agonist stimulation of these receptors exerts a significant lipogenic action in the liver. In $CB1^{+/+}$ mice, administration of the potent CB1 agonist HU210 upregulated expression of the lipogenic gene transcription factor, sterol response element-binding protein 1c (SREBP-1c), and its target enzymes acetyl-coA carboxylase-1 (ACC1) and fatty acid synthase (FAS). These rimonabant-reversible changes were accompanied by an increase in *de novo* fatty acid synthesis. Additional analysis revealed that in comparison to $CB1^{+/+}$ mice, the level of SREBP-1c expression in liver and adipose tissue was reduced in diet-induced obesity-resistant $CB1^{-/-}$ mice. Importantly, in wild type mice, obesity induced by overconsumption of a high fat diet was found to elevate liver anandamide levels (through reduction of its enzymatic breakdown by FAAH), upregulate hepatic CB1 receptors, and promote hepatic fatty acid synthesis [154]. These changes were prevented by rimonabant and absent in $CB1^{-/-}$ animals, suggesting a key role for hepatic endocannabinoids in the development of obesity.

Kunos' group have also proposed that CB1-mediated regulation of FAS within the brain provides for a common pathway linking central endocannabinoid regulation of appetite and peripheral metabolic processes [154]. In support of this notion is the fact that lipid metabolism in feeding-related hypothalamic neurons is sensitive to nutrient availability, and that inhibition of FAS can suppress eating [19,271]. HU210 treatment in wild type mice upregulated hypothalamic SREBP-1c and FAS mRNA, effects which were reversed by rimonabant. Similar effects were detected in 24-h fasted rats after restoration of food, but not in non-deprived, *ad libitum* fed animals [154]. From these results it was concluded that lipogenic gene expression in the

hypothalamus is linked to the control of food intake. However, since no effect of fasting on hypothalamic SREBP-1c/FAS expression was detected in fasted animals killed without re-feeding, it is difficult to conclude what the precise nature of the controlling relationship might be. Possibly, hypothalamic SREBP-1c/FAS activity is linked to factors that respond to energy intake after fasting, rather than directly controlling feeding behaviour.

As already discussed, there is evidence of regulation of endocannabinoid function by the adipokine leptin. Recent work also supports interaction with another adipokine, adiponectin. Specifically, there are parallels between the effects of rimonabant and adiponectin, suggesting a possible mechanism whereby body weight is reduced by the antagonist. Adiponectin expression varies inversely with adiposity in animals and humans. Like rimonabant, adiponectin regulates hyperglycaemia, hyperinsulinemia and fatty acid oxidation, and can reduce the body weight of obese animals through a food intake-independent mechanism [272,273]. Bensaid *et al*. [269] examined the effects of daily rimonabant administration in obese Zucker rats. Again, the drug produced an initial, short-lasting anorexia and marked, persistent weight reduction. After 4 days of treatment, adiponectin mRNA expression was modestly, but significantly increased. More marked effects were apparent with longer periods of treatment, with a maximal (1.5-2.0 fold) increase in adiponectin mRNA expression evident after 10 days. A similar action was also observed in lean Zucker rats, but to a lesser extent and with a slower rate of onset. The rimonabant-induced changes to adiponectin mRNA expression paralleled weight loss and normalization of hyperinsulinemia. *In vitro* studies with cultured mouse adipocytes showed that rimonabant stimulation of adiponectin mRNA expression was CB1 receptor mediated, since no effect of the drug was apparent in adipocytes from CB1 knockout mice. Poirier *et al*. obtained similar results with chronic rimonabant treatment over 10 weeks in dietary obese mice maintained on a high fat diet: persistent weight loss was associated with a small, but significant increase in serum adiponectin levels [193].

Liu and colleagues [274] have proposed that adiponectin may affect body weight by promoting peripheral fatty acid oxidation in muscle. They detected increased thermogenesis in obese Lep^{ob}/Lep^{ob} mice treated chronically with rimonabant, which was associated with increased glucose uptake by isolated soleus muscle from these animals. These data suggest that the sustained effects of the drug on body weight (outlasting its anorectic action) involve increased energy expenditure; possibly by stimulation of efferent sympathetic activity, since rimonabant has been shown to increase noradrenalin outflow in the anterior hypothalamus [275]. Liu *et al*. also suggested that rimonabant-induction of adiponectin expression, with a consequent reduction in free fatty acids, might drive the glucose-fatty acid cycle and account for the increased glucose uptake and improved hyperglycaemia [274].

Given the ability of rimonabant to affect glucose homeostasis and to improve insulin resistance, it is notable that CB1 and CB2 receptors have been detected in the endocrine pancreas [276]. Although CB1 appear to be mainly expressed in non-β cells, Juan-Pico and colleagues have shown that CB1 activation can suppress insulin secretion. Additionally, 2-AG also acts *via* CB2 receptors to regulate Ca^{2+} signaling in β-cells and decrease insulin secretion. Moreover, it is proposed that CB1 receptors in α-cells may be involved in the regulation of glucagon release.

In direct support of an energetic account of rimonabant's actions on weight is the recent report by Jbilo and colleagues who examined the consequences for adipocyte gene expression of long-term treatment with the drug in dietary obese mice [277]. After 40 days rimonabant produced a 50% reduction in fat mass (18% overall body weight reduction). This loss was associated with enhanced lipolysis, through the induction of enzymes of the β-oxidation and tricarboxylic acid (TCA) cycles in adipose tissue. Rimonabant also induced genes for enzymes that contribute to glycogen and amino acid metabolism and consequently, the regulation of energy expenditure. The drug also upregulated the expression of several glycolytic enzymes that are critical regulators of glucose metabolism. Additionally, adiponectin expression was also induced by rimonabant. Importantly, similar gene modulation was apparent in CB1 knockout mice fed a high fat diet. The authors concluded that rimonabant reduces adipose levels by increasing fatty acid oxidation and energy expenditure, and adipose tissue may the specific target for the drug's peripheral anti-obesity action.

Further indications of how energy storage and feeding behaviour may be linked through cannabinoid mechanisms comes from a recent study of AMP-activated protein kinase (AMPK). AMPK is an enzyme proposed to function as a fuel sensor, contributing to energy balance regulation at both cellular and whole body levels [278,279]. We found that both THC and 2-AG stimulated AMPK activity in the hypothalamus, while inhibiting AMPK activity in the liver and adipose tissue [280]. Given the proposed role of AMPK, these observations may provide important evidence of interactions between this enzyme and the orexigenic actions of cannabinoids. Thus, cannabinoids could potentially increase appetite by central AMPK stimulation, or by facilitating the restorative actions of AMPK as the hypothalamus senses fuel deprivation. By contrast, peripheral inhibition of AMPK by cannabinoids may lead to fat storage. The combined effect of both central and peripheral AMPK activation could therefore be both increased food intake and increased lipid deposition in adipocytes.

CANNABINOIDS IN HUMAN OBESITY AND EATING DISORDERS

Despite the topicality of endocannabinoids in relation to appetite and body weight regulation, and the speed of developments in animal models of obesity, there has so far been little investigation of changes to endocannabinoid systems in human obesity. However, three recent studies suggest that further enquiry may be instructive. Monteleone *et al*. [281] examined plasma levels of anandamide and 2-AG in women with anorexia nervosa, bulimia nervosa or binge eating disorder. They found that plasma levels of anandamide were significantly elevated in both anorexics and women with binge eating disorder, but not in bulimic patients. No significant alterations to 2-AG levels were detected in any of the groups. Anandamide and 2-AG levels were not reliably correlated with the severity of psycho-

logical symptoms or duration of illness (which reflects the wide distribution of plasma levels within each group, and the considerable overlap between groups); nor was there any significant relationship between levels of the two endocannabinoids. Additionally, circulating anandamide levels showed a significant inverse correlation with plasma leptin concentrations in healthy controls, anorexics and women with binge-eating disorder.

These findings suggest the possibility of some derangement in the production of anandamide in women with these particular disorders, and the authors tentatively proposed that their data reflected endocannabinoid mediation of the rewarding aspects of aberrant eating behaviours. The full significance of these findings will depend on verifying the source of anandamide that was measured, and the extent to which plasma levels reflect altered endocannabinoid regulation in critical regulatory systems. Particularly difficult to interpret is the elevation of anandamide in both restricting, underweight anorexics and obese, overconsuming binge-eaters. The authors suggested that similar changes in each population reflected, respectively, reduced leptin in anorexia nervosa patients and leptin insensitivity in the binge eaters as a consequence of associated obesity. Based on the ability of leptin to inhibit anandamide in animal models [199], it was suggested that increased levels of this substance in anorexics was secondary to leptin deficiency [281].

These complexities are multiplied by the finding that there may be genotypic differences between subtypes of anorexia nervosa. Specifically, Siegfried *et al*. [282], examined the frequency of a polymorphism of the human CB1 (*CNR1*) gene in the parents of restricting and binge-purging anorexic nervosa patients. Analysis of AAT triplet repeat marker (located downstream of the coding region) revealed preferential transmission of different alleles of the cannabinoid receptor gene according to patient symptomatology: a 13 repeat allele was preferentially transmitted in restricting anorexics, while a 14 repeat form was transmitted in bingeing/purging individuals. The authors suggest that the specific alleles do not necessarily increase an individual's susceptibility to developing anorexia nervosa, but may modify the form of its expression.

A single study by Sipe *et al*. [283] revealed obesity-related variations in a naturally occurring missense polymorphism (cDNA 385 C−A) in the gene encoding FAAH, the primary enzyme for inactivation of anandamide. This polymorphism was assessed because of its previously reported linkage to 'reward disorders' in drug abuse, with the hypothesis that reward processes are common to drug use and overconsumption of food. A homozygous FAAH 385 A/A genotype was significantly associated with overweight and obesity in white and black individuals, but not in a small group of Asians. Overall, median body mass index (BMI) was significantly greater in the FAAH 385 A/A genotype group compared to heterozygote and wild-type groups, with a higher frequency of the FAAH 385 A/A genotype as BMI increased: the strongest relationship was observed in the white cohort. The authors concluded that this missense polymorphism could indicate an endocannabinoid risk factor in the development of overweight and obesity. The underlying mechanism possibly being related to reduced FAAH expression and activity [284]. By extrapolation, concomitant accentuation of endocannabinoid activity might render individuals carrying this polymorphism more susceptible to the rewarding properties of palatable, energy-dense foods, and so more likely to overconsume.

CB1 RECEPTOR ANTAGONISTS AS ANTI-OBESITY TREATMENTS: CLINICAL TRIALS

In parallel with studies in animal models on the feeding and metabolic consequences of CB1 antagonist administration has been the clinical assessment of rimonabant in relation to its potential in reducing cardiovascular risk factors; particularly the abdominal obesity, dyslipidemia, and insulin resistance associated with the metabolic syndrome. Findings from 2-year, Phase III clinical trials with rimonabant (Acomplia) have now been published (Rimonabant in Obesity-Europe [285], RIO-North America [286]), while other studies are underway (RIO-Lipids and RIO-Diabetes). The data so far indicate that the drug can have positive benefits in relation to obesity and its associated disorders (for a review of pre-publication data, see [287]), and in June 2005, Sanofi-Aventis filed a new drug application for rimonabant with the US Food and Drug Administration.

The typical protocol of these studies involves administration of 5 or 20 mg/d of rimonabant to obese patients of BMI >27 kg/m^2 with co-morbidities such as dyslipidaemia and hypertension. Patients were recommended a daily energy intake level 600 kcal/d lower than their pre-treatment baseline. Results from the first of these trials (RIO-Lipids) were presented in March 2004 to the American College of Cardiology. The data indicated that over 1 year patients treated with 20 mg/d lost an average of 8.6 kg – significantly more than the 2.3 kg loss in the placebo group. Almost 75% of patients in the rimonabant group lost >5% body weight, relative to 42% with placebo. Greater than 10% weight loss was observed in 44% of rimonabant-treated patients, compared to 10% of controls. Additionally, 20 mg/d rimonabant produced: a waist circumference reduction of 9.1 cm; a 23% increase in HDLc; a 15% reduction in triglycerides; a reduction in atherogenic LDL particles, and improved insulin sensitivity. Additionally, in a subgroup of patients treated with 20 mg rimonabant, plasma leptin was reduced, while adiponectin levels were significantly increased.

First year RIO-Diabetes results were reported in June 2005 at the Congress of the American Diabetes Association. As a measure of long term blood glucose levels HbA1c levels were assessed. Of patients with pathologically elevated baseline levels (>7%), 53% treated with 20 mg reduced their HbA1c levels below 7% by the end of the study (versus 27% of patients with placebo), although the overall reduction in rimonabant-treated patients was only 0.7% compared to placebo. An average weight loss of 5.3 kg was observed with 20 mg/d rimonabant, compared to 1.4 kg in the placebo group. After rimonabant, HDL-cholesterol showed a significant increase of 15% (versus 7% with placebo), and triglycerides were reliably reduced by 9% (compared to 7% increase with placebo).

The first peer-reviewed report of a clinical trial (RIO-Europe) confirmed the earlier reports of beneficial changes in metabolic and cardiovascular risk factors [285]. In overweight and obese patients maintained on 20 mg/d for 1 year, rimonabant produced a mean weight reduction of 6.6

kg (to a maximum of ~ 10 kg) compared to only 1.8 kg after placebo (for the intention to treat group; weight loss reached an average of 8.6 kg in completers). Significantly more of the rimonabant-treated group achieved greater than 10% weight loss (67% of completers at 20mg/d, compared to 30% in placebo). The drug significantly increased HDL-cholesterol by 22% compared to a 13% rise in the placebo group (without any significant change in LDL or total cholesterol). Additionally, 20 mg/d rimonabant significantly reduced waist circumference (8.5 cm compared to 4.5 cm in controls), lowered plasma triglyceride levels by 6.8% (compared to increased levels in placebo and 5 mg/d rimonabant groups), corrected hyperglycaemia, reduced plasma insulin levels and countered insulin resistance. The prevalence of metabolic syndrome was reduced by more than 50% in the rimonabant group. No interaction between sex and weight loss, metabolic parameters or waist circumference were noted; and no significant changes were detected in relation to systolic and diastolic blood pressure.

Similar results were reported in relation to the RIO-North America study which also examined the effects of rimonabant over 2 years [286]. Relative to the placebo group, at the end of year 1 patients receiving 20 mg/d rimonabant had significantly greater weight loss (−6.3 kg versus −1.6 kg), reduced waist circumference (−6.1 cm versus −2.5 cm), lower triglyceride levels (−5.3% versus 7.9%), a greater increase in HDL- cholesterol (12.6% versus 5.4%), and improved insulin resistance. As in the RIO-Europe study, there was no improvement in systolic or diastolic blood pressure. During year 2, patients who continued on rimonabant maintained the weight loss achieved during year 1 (without any further weight loss) and the favourable changes in cardiometabolic risk factors. The authors noted that levels of HDL-cholesterol continued to rise in the rimonabant group; although it should be noted that a parallel rate of increase was apparent in the placebo group over the same period. Over 2 years, reliable improvements in insulin resistance over baseline were no longer evident. After 2 years, mean weight loss from baseline was 7.4 kg, and overall waist circumference reduction was 5.0 cm (compared to 2.2 cm in the placebo group). By contrast, patients who were switched from rimonabant to placebo during year 2 fully regained lost weight and girth, reaching placebo levels after 9-10 months.

Overall, the general efficacy of rimonabant in counteracting the symptoms of metabolic syndrome is comparable to that for the currently prescribed appetite suppressant, sibutramine (for comparative analysis see [287]). However, in each of these studies, it has been argued that some of rimonabant's effects on specific parameters are separable from weight loss. For example, Van Gaal *et al.* [285] claimed that as much as 40% of the drug's effect on HDL-cholesterol and 65% of the action on triglycerides were independent of weight loss. Pi-Sunyer's [286] group argued that rimonabant's effects on HDL-cholesterol, triglycerides, fasting insulin and insulin resistance were approximately double those likely to result from the observed weight loss alone. These weight-independent effects were attributed to the proposed action of rimonabant to stimulate adiponectin (although no data on levels in the trials were presented). However, the real contribution and extent of adiponectin in rimonabant's pharmacological profile remain to be determined – and must be considered in relation to the other important metabolic effects of rimonabant noted above.

In each of the RIO trials, rimonabant was reported to be generally well-tolerated. However, elevated levels of nausea, anxiety, depression and insomnia may be of concern given the known actions of CB1 agonists and antagonists in animal models and the likely role of endocannabinoid systems in emesis, emotionality and sleep function [204,205,221,288, 289]. Clearly, detailed analysis of rimonabant's actions on the psychological and behavioural aspects of appetite in people would be very informative. A further trial examining these aspects of rimonabant's action (REBA-UK) may provide the necessary information in the near future.

CONCLUSION

The data reviewed here support an important role of endocannabinoids and CB1 cannabinoid receptors in the processes that normally regulate appetite and eating behaviour. The additional involvement of cannabinoid-sensitive mechanisms in the peripheral regulation of adiposity and energy balance indicates that endocannabinoid systems represent a potentially unique target for the treatment of disorders of appetite and body weight regulation. The combined ability of rimonabant to alter fat metabolism and glucose utilisation, promote weight loss, and to suppress food intake suggests that CB1 receptor antagonists may be powerful tools in the treatment of obesity, metabolic syndrome, cardiovascular disease, type 2 diabetes and atherogenesis.

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ABBREVIATIONS

GPCR = G protein-coupled receptors

THC = Δ^9-tetrahydrocannabinol

NADA = *N*-arachidonoyldopamine

NAPE = N-acyl-phosphatidylethanolamine

PLD = Phospholipase D

2-AG = 2-arachidonoylglycerol

DAGs = *Sn*-1-acyl-2-arachidonoylglycerols

FAAH = Fatty acid amide hydrolase

MAGL = Monoacylglycerol lipase

COX-2 = Cyclooxegenase-2

EMT = Endocannabinoid membrane transporter

THCV = Tetrahydrocannabivarin

DMV = Dorsal motor nucleus of the vagus

NTS = Nucleus tractus solitarius

VTA = Ventral tegmental area

AcbSh = Nucleus accumbens shell

NPY = Neuropeptide Y

α-MSH = α-melanocyte stimulating hormone
MCR = Melanocortin receptor
CART = Cocaine amphetamine-regulated transcript
PVN = Paraventricular nucleus of the hypothalamus
CRH = Corticotropin-releasing hormone
MCH = Melanin-concentrating hormone
CCK = Cholecystokinin
SREBP-1c = Sterol response element-binding protein 1c
ACC1 = Acetyl-coA carboxylase-1
FAS = Fatty acid synthase
cAMP = Cyclic adenosine monophosphate
AMPK = AMP-activated protein kinase
BMI = Body mass index
HDLc = High-density lipoprotein cholesterol
LDLc = Low-density lipoprotein cholesterol

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