REVIEW

Cannabinoids for clinicians: the rise and fall of the cannabinoid antagonists

Helen Butler and Márta Korbonits

Department of Endocrinology, Barts and the London School of Medicine, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK

(Correspondence should be addressed to M Korbonits; Email: m.korbonits@qmul.ac.uk)

Abstract

The endocannabinoid system has emerged as a significant player in the control of energy balance and metabolism, through its direct central and peripheral effects, as well as via its interaction with other appetite-regulating pathways. There is mounting evidence that the endocannabinoid system is overactive in obesity and were it possible to safely dampen-down the elevated endocannabinoid tone, lipid and carbohydrate profiles could be improved and weight loss induced. The series of randomised clinical trials showed reproducible beneficial effects on weight, HbA1c and lipid parameters, in addition to other cardiovascular risk factors. However, to date, clinical developments have been halted because of psychiatric side effects. Although recent evidence has highlighted the importance of an appetite-independent, peripheral mode of action, it is still unclear whether selectively blocking the peripheral system could potentially solve the problem of the central side effects, which thus far has led to the demise of the cannabinoid antagonists as useful pharmaceuticals. In this concise review, we summarise the data on the metabolic effects of the cannabinoid pathway and its antagonists.

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Introduction

The endocannabinoid system is a complex physiologic system that is highly relevant in the control of energy balance and metabolism (1). Upon stimulation, it increases food intake and weight gain, promotes lipogenesis and impairs glucose tolerance (2). There is growing evidence that the endocannabinoid system is overactive in obesity (3, 4), and thus targeting and suppressing the system could result in a potential pathway by which to treat obesity, type 2 diabetes and the metabolic syndrome. Despite the early promising results of the cannabinoid antagonists, the drug's side effect profile regarding depression and suicidal risk has been deemed unsafe, and thus, to date, all preparations have been withdrawn from trials or clinical practice. Here, we discuss the discovery, physiology and mechanism of action of the endocannabinoid system, along with its potential for manipulation in the treatment of obesity.

The discovery and physiology of the endocannabinoids

The plant *Cannabis sativa* has been used to promote caloric intake by enhancing appetite for hundreds of years (5, 6). Despite knowledge of its medical benefits for centuries, it was not until 1964 that the psychoactive component of cannabis was isolated as Δ-9-tetrahydrocannabinol (7), which subsequently led to the discovery and cloning of two specific $G_{i/o}$ protein-coupled cannabinoid receptors, CB_1 (8) and CB_2 (9). Both receptors are expressed in the CNS, as well as in peripheral tissues. CB_1 was found to be one of the most prevalent G protein-coupled receptors in the mammalian brain, while CB_2 was shown to have prominent roles in immune and haematopoietic cells, as well as osteoblasts and osteoclasts (10–13). The discovery of specific cannabinoid receptors implied that endogenous ligands capable of activating these receptors must exist. Anandamide and 2-arachidonoylglycerol (2-AG) are the two most widely studied 'endocannabinoids'. They are not stored in vesicles like other neurotransmitters, but produced on demand by Ca^{2+}-induced enzymatic cleavage from phospholipid precursors (14). CB_1 receptors are often localised on pre-synaptic neurons, which suggest retrograde signal transmission (15) (Fig. 1), whereby the endocannabinoids usually act to reduce neuronal excitability via inhibitory effects on voltage-gated Ca^{2+} channels and the activation of K^+ channels (16) (Fig. 2). In addition to CB_1 and CB_2, several other receptors were shown to be targets of endocannabinoids, including the transient receptor potential cation channel, subfamily V, member 1 ($TPRV_1$) (17), a novel orphan cannabinoid receptor GPR55 (18) and additional unidentified endothelial and cardiac receptors, which may mediate endocannabinoid-induced cardiovascular effects (19, 20).
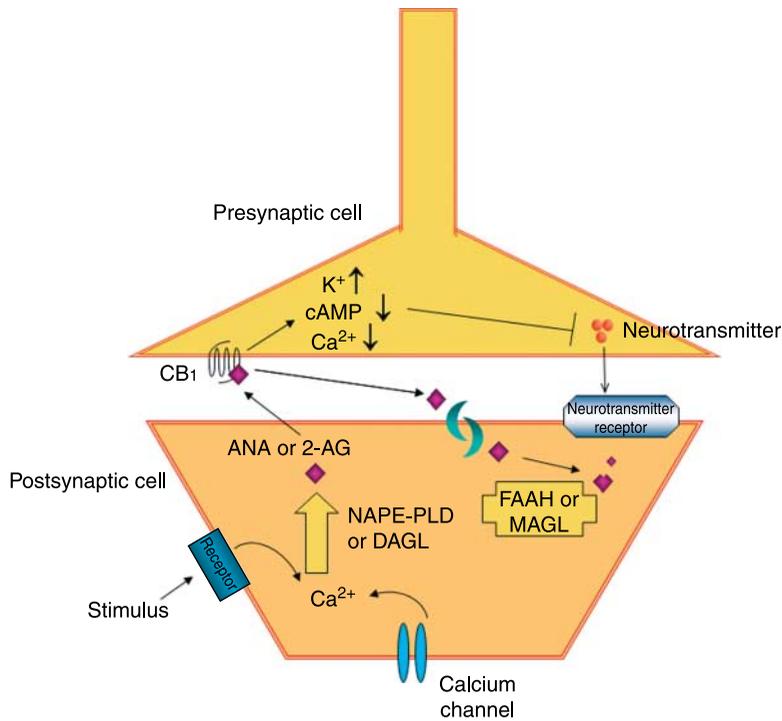
Figure 1 Upon stimulation of the postsynaptic cell, an influx of intracellular calcium results in the activation of *N*-acylphosphatidylethamolamine hydrolysing phospholipase D (NAPE–PLD) and diacyl–glycerol lipase (DAGL-α and -β) and release of endocannabinoids (e.g. anandamide (ANA) or 2-arachidonoylglycerol (2-AG)) from the postsynaptic terminal. The endocannabinoid crosses the synaptic cleft and binds to its receptor on the presynaptic terminal. Cannabinoid receptor activation often results in blocked neurotransmitter release from the presynaptic neuron. The endocannabinoids are taken up by the postsynaptic cell via a method of unspecified transport (green crescents) and are finally inactivated and degraded by fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL) (83, 84).

The cannabinoids have wide ranging effects on various systems including mood, cognition and reward, immune, gastrointestinal and reproduction, sleep, neuroprotection, bone and cardiovascular function, in addition to appetite, lipid and carbohydrate metabolism (15). The orexigenic effect of the endocannabinoids is mediated via activation of AMP-activated protein kinase (AMPK) (21–23). AMPK plays a central role in the control of energy homeostasis both at an individual cellular level and that of the whole body via its appetite-stimulating effects in the hypothalamus. It can sense the cellular 'energy status' and upon activation causes the cell to switch from ATP consumption to ATP production. AMPK is known to be influenced by various stimuli such as leptin, ghrelin, adiponectin and α-melanocortin-stimulating hormone, as well as metformin and glitazones (24).
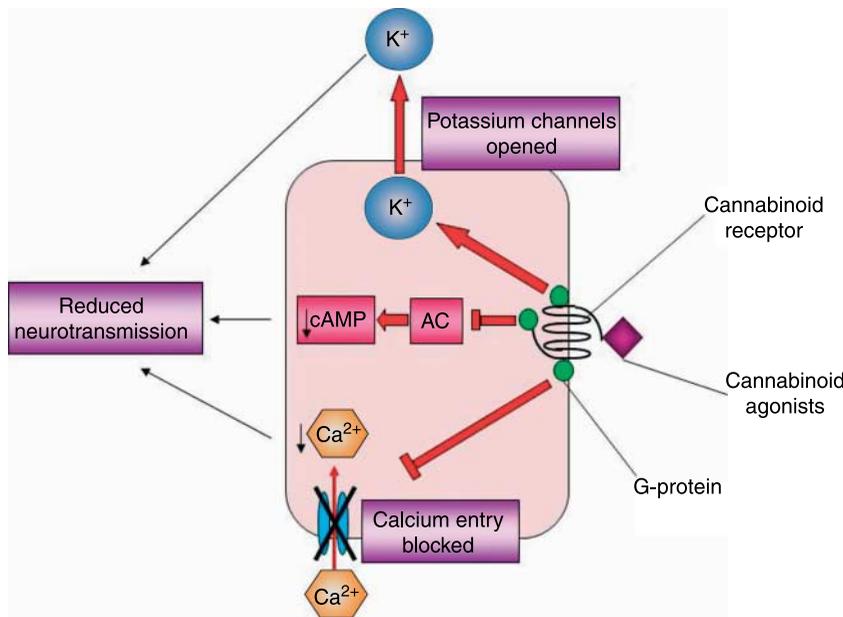
Figure 2 Cannabinoid receptor activation often leads to reduced cellular neuro-transmission by blocking Ca^{2+} entry, via hyperpolarisation, as a result of K^+ channel activation, or by reduced cAMP generation.

Interaction with other hormones and systems

It seems that cannabinoids interact with a number of hormonal systems and possibly mediate their effects. Data for heterodimerisation of the receptor with the orexin (25), type 2 dopamine (26) and opioid receptors (27) have been shown, and possible other partners have been suggested, such as the ghrelin receptor (28) (Table 1).

Mode of action: central versus peripheral

Initially, it was thought that the endocannabinoid system acted solely centrally through its effects on appetite and food intake. In support of this, the CB_1 receptor is widely prevalent centrally (8, 42), and central administration of anandamide into the ventromedial nucleus causes hyperphagia in rats (22). However, the endocannabinoids not only act centrally but also peripherally in the control of energy balance and metabolism. CB_1 receptors have been found peripherally on the nerve terminals of gastrointestinal tract neurons (43), liver (44), skeletal muscle (45), pancreas (46) and in adipose tissue (47). Upon pair feeding of CB_1 knockout ($CB_1^{-/-}$) mice with normal littermates, wild-type mice gained weight, while the $CB_1^{-/-}$ did not, despite the same caloric intake (48). Similarly, chronic injection of a CB_1 receptor antagonist in obese rodents caused significant weight loss, which was sustained despite only a transient decrease in caloric intake (49). A human study showed an increase in caloric intake and weight gain in volunteers who smoked cannabis when compared with controls (50); interestingly, although the weight of the participants continued to increase throughout the 3-week study, the increased food intake subsided after just a few days.

This suggests that the cannabinoids increase weight by methods other than solely stimulating appetite. In addition, food deprivation leads to a sevenfold increase in intestinal levels of anandamide, and this effect is reversed upon refeeding. When the neurotoxin capsaicin was used to destroy the sensory afferent fibres of the gut, the effects of peripherally administered anandamide were abolished. This is supportive of a peripheral mechanism of action, whereby anandamide acts on peripheral CB_1 receptors located on the sensory nerve terminals of the gut to create its hyperphagic effect (51). Furthermore, experimental evidence on adipose tissue has shown that, in addition to CB_1 receptors, enzymes regulating the biosynthesis and degradation of the endocannabinoids are present, and the levels of endocannabinoids in adipose tissue were found to be similar to those of the brain (52). This is probably due to an increase in the fat oxidation rate. First, this is supported by a reduction in the respiratory quotient. Secondly, experiments in adipocytic cultures have shown that chronic CB_1 blockade reduces adipose mass by increasing oxidation rate, via the induction of enzymes of β-oxidation and the Krebs cycle (53). Thirdly, in the opposite situation, adipocyte cultures treated with cannabinoid agonists show lipoprotein lipase activation (2) and decreased expression of adiponectin (54), which cause triglyceride re-esterification and reduce fatty acid oxidation. Most of these effects can be explained by the inhibitory effect of cannabinoids on liver and adipose tissue AMPK activity (23). Osei-Hyiaman *et al.* investigated the role of the liver as a target for the peripheral regulation of energy metabolism by the endocannabinoids. When given a high-fat diet, the density of CB_1 receptors increased, as did levels of hepatic anandamide. Upon activation, *de novo* fatty acid synthesis occurred (48) due to an increase in fatty acid synthase and acetyl-CoA carboxylase, thus promoting insulin resistance and hepatic steatosis (55).

Table 1 Interaction with other hormones and systems.

Interacting partners	Effect	Reference
Leptin	• Leptin reduces endocannabinoid levels in the hypothalamus • Leptin-deficient animals reduce food intake in response to a CB_1 antagonist • Leptin-deficient animals have high endocannabinoid levels • Anorexia nervosa patients have high circulating endocannabinoid levels	(30–33)
Ghrelin	• Ghrelin increases endocannabinoid levels in the hypothalamus • Intact CB_1 is necessary for ghrelin appetite and AMPK effects in the hypothalamus	(28)
Adiponectin	• CB_1 antagonist increases adiponectin levels • The beneficial effects of CB_1 antagonist are partly (but not fully) via adiponectin	(30, 34)
NPY, αMSH	• Anandamide increases hypothalamic neuropeptide Y, an effect inhibited by cannabinoid antagonists • α-MSH and the cannabinoid system have been shown to decrease food intake synergistically	(35–36)
Orexin	• Orexin receptor, OX1R, was shown to heterodimerise with the CB_1 receptor, leading to increased orexin effects	(25, 37)
Glucocorticoids	• Glucocorticoids release endocannabinoids in the hypothalamus	(38, 39, 82)
Neurotransmitters	• The endocannabinoid system influences the release of various neurotransmitters, including GABA, noradrenaline, dopamine, glutamate and acetylcholine • A direct interaction of dopamine receptors and CB_1 has been suggested	(29, 40, 41)

Treatment with a CB_1 antagonist has been shown to reduce hepatic steatosis in rats (56), while results of treatment with rimonabant for non-alcoholic fatty liver disease in humans have been promising (57).

Hyperactivity of the endocannabinoid system in obesity

Both animal and human data show that the endocannabinoid system is up-regulated in obesity (58). A significantly higher amount of 2-AG was found in visceral fat in obese and overweight individuals when compared with normal-weight controls (54). A study on obese, postmenopausal women showed raised 2-AG and anandamide levels, along with reduced fatty acid amide hydrolase (FAAH) expression compared with control subjects (59), suggesting that impaired degradation of endocannabinoids could play a role. Further data to support the importance of endocannabinoid degradation showed that a missense polymorphism in FAAH was found to correlate with body mass index (BMI) in obese patients (60). Those with the polymorphism had only half the FAAH enzymatic activity of controls, thus providing substantial evidence for an upregulation of the endocannabinoid system in obesity due to, at least in part, a defect in the mechanism of degradation (60). However, these data were not reproduced in a similar study, which included over 5000 patients (61). Polymorphisms of the *CNR1* gene encoding the CB_1 receptor have also been shown to be associated with obesity (62).

Manipulating the system

The hyperactive endocannabinoid system in obesity has been targeted, using CB_1 receptor antagonists, in an attempt to attenuate endocannabinoid signalling. Animal studies have shown that by blocking the CB_1 receptor, both food intake and weight are significantly reduced (22, 49, 63, 64). Following on from these promising results, Sanofi-Aventis commenced human clinical trials to test the safety and efficacy of rimonabant in the treatment of obesity (65). The rimonabant in obesity (RIO) trials, with over 6600 participants, composed of four separate trials: RIO North America (66), RIO Europe (67), RIO Diabetes (68) and RIO Lipids (69), all published in distinguished journals. Inclusion criteria for the RIO Europe and RIO North America trials included a BMI > 30 kg/m^2 or a BMI > 27 kg/m^2 with obesity-induced disease (66, 67). The RIO Lipids trial involved hyperlipidaemic, overweight and obese participants who had previously had no medical therapy, while the RIO Diabetes trial included type 2 diabetic patients on monotherapy, who were also overweight or obese. At 1-year follow-up, the results of the randomised, double-blind, placebo-controlled trials showed a placebo-corrected weight loss of 4.7 kg (range 4.1–5.4)

in the 20 mg group in RIO North America and 4.8 kg (3.9–5.7) in RIO Europe. RIO Lipids reported a weight loss of 5.4 kg (4.6–6.3), and RIO Diabetes result was 3.9 kg (3.2–4.6) (66–69). Furthermore, HbA1c and triglyceride levels decreased, while high density lipoprotein cholesterol increased. Waist circumference was also measured in the RIO trials and resulted in statistically significant reductions in the 20 mg groups (6.1, 6.5, 7.1 and 5.2 cm respectively) compared with placebo (2.5, 2.4, 2.4 and 1.9 cm respectively). Further studies commenced including type 2 diabetic patients with no previous drug treatment (70), patients on established diabetic treatment and patients with cardiovascular risks factors were specifically followed. These studies also resulted in positive outcomes (71). The data suggested that rimonabant was a promising agent, and thus it was licensed in the European Union (EU) as an anti-obesity drug and approved by National Institute for Health and Clinical Excellence for use in the UK. Its indications were as an adjunct to a healthy diet and exercise in obese patients (BMI > 30 kg/m^2) and for overweight adults (BMI > 27 kg/m^2) with associated risk factors, such as type 2 diabetes or dyslipidaemia (NICE: National Institute for Health and Clinical Excellence: Rimonabant for the treatment of overweight and obese patients: http://www.nice.org.uk 2007).

However, a high incidence of adverse effects was reported in the RIO trials' 20 mg groups. The most common side effects reported were headache, nausea, anxiety and depressed mood (69). Withdrawal rates were 15.0, 14.5, 12.8 and 15.0% respectively in the four trials, compared with placebo withdrawal rates of 7.0, 9.2, 7.2 and 5.5% (72). Because of the psychotropic nature of cannabis, psychiatric side effects are biologically plausible, and indeed 26% of participants in the 20 mg groups reported psychiatric symptoms. These included depression, anxiety, sleep disorders and suicidal ideation (70). However, data suggest that in real-life clinical practice, $\sim 50\%$ of individuals seeking treatment for obesity also suffer from depression, making it hard to ascertain whether or not reports of depression were attributable to the drug or not (73, 74).

As a result of the psychiatric side effect profile, the US Food and Drug Administration declined permission for rimonabant, and in October 2008, rimonabant was also suspended across the EU. The European Medicines Agency (EMEA) disputed its psychiatric safety, due to an increased risk of depression and significant suicide risk. Their analysis showed an approximately doubled risk of psychiatric disorders, including depression, anxiety, sleep disturbance and suicidal ideation, in those taking rimonabant when compared with placebo. Since the withdrawal of rimonabant, other CB_1 antagonist drugs have also been halted in the process of development. Taranabant (Merck & Co.) has been shown to induce chronic weight loss in diet-induced obese rats (75, 76). In a human trial, treatment with 12 mg taranabant resulted in a significant increase in

resting energy expenditure, most likely due to an increase in the fat oxidation rate, supported by a reduction in the respiratory quotient when taking the drug (77). Although the drug reached phase-III human obesity trials, they were stopped in October 2008, due to a high level of central side effects, including anxiety and depression (EMEA. The European Medicines Agency recommends suspension of the marketing authorisation of Acomplia: http://www.emea.europa.eu 2008).

Rimonabant has high affinity binding for the CB_1 receptor and acts as a full inverse agonist. An alternative would be the use of a partial agonist, which would decrease cannabinoid receptor activation as well as preventing the psychiatric side effects evident when completely blocking CB_1 (78). Partial agonists are reported to have a lower prevalence of adverse effects than either antagonists or inverse agonists, without reducing the efficacy of the drug (79). An example of such a drug with the potential for an anti-obesity effect is the $5-HT_6$ ligand, E-6837 (80). The use of partial agonists at pharmacological targets in the endocannabinoid system may produce the significant weight loss results of the cannabinoid antagonists, but without the psychiatric side effects. Data is available on a neutral CB_1 cannabinoid antagonist, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4,-triazole-LH21 (81). Its lack of inverse agonist properties and low permeation of the blood–brain barrier resulted in an improved side-effect profile when compared with inverse agonists.

Conclusion

The hyperactive endocannabinoid system established in obesity has been supported with strong evidence from both animal and human experiments, with the argument for a genetic basis especially interesting. Breaking down the system and blocking the endocannabinoid pathway at any level could potentially diminish its excessive activity and promote weight loss. The peripheral actions of the system are intriguing, and selectively blocking the peripheral receptors may hold the solution to preventing the central side effects, which thus far have led to the downfall of the cannabinoid antagonists in therapeutic medicine. However, it is currently unclear whether the beneficial effects on weight, as well as lipid and carbohydrate metabolism, would be upheld in this case, as convincing data of direct central regulation of peripheral metabolism are emerging.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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