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Mini-review

The endocannabinoid system, eating behavior and energy homeostasis: The end or a new beginning?

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ABSTRACT

The endocannabinoid system (ECS) consists of two receptors (CB_1 and CB_2), several endogenous ligands (primarily anandamide and 2-AG), and over a dozen ligand-metabolizing enzymes. The ECS regulates many aspects of embryological development and homeostasis, including neuroprotection and neural plasticity, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, and the focus of this review: hunger, feeding, and metabolism. This mini-review summarizes the main findings that supported the clinical use of CB1 antagonists/inverse agonists, the clinical concerns that have emerged, and the possible future of cannabinoid-based therapy of obesity and related diseases. The ECS controls energy balance and lipid metabolism centrally (in the hypothalamus and mesolimbic pathways) and peripherally (in adipocytes, liver, skeletal muscle and pancreatic islet cells), acting through numerous anorexigenic and orexigenic pathways. Obese people seem to display an increased endocannabinoid tone, driving CB_1 receptor in a feed-forward dysfunction. Several CB_1 antagonists/inverse agonists have been developed for the treatment of obesity. Although these drugs were found to be efficacious at reducing food intake as well as abdominal adiposity and cardiometabolic risk factors, they resulted in adverse psychiatric effects that limited their use and finally led to the end of the clinical use of systemic CB_1 ligands with significant inverse agonist activity for complicated obesity. However, the existence of alternatives such as CB_1 partial agonists, neutral antagonists, antagonists restricted to the periphery, allosteric modulators and other potential targets within the ECS indicate that a cannabinoid-based therapy for the management of obesity and its associated cardiometabolic sequelae should remain open for consideration.

Contents

1. Introduction

The cannabinoid type-1 receptor (CB_1) has recently emerged as a promising target for modulating energy balance and thereby management of obesity, a high-prevalence disease with a high rate of

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associated co-morbidities. Unfortunately, part of the initial enthusiasm faded as significant psychiatric side effects were reported, leading the U.S. Food and Drug Administration (FDA) to disapprove the clinical use of the CB_1 antagonist/inverse agonist rimonabant in the USA, and the European Medicines Agency (EMEA) to recommend marketing suspension of Acomplia®, the commercial name of the drug. This has engendered a great deal of controversy both in the research community and the pharmaceutical industry regarding the future of a cannabinoid-based therapy for management of obesity and/or related diseases. This mini-review provides a comprehensive overview of this topic, reviewing the main findings that initially supported the clinical use of CB1 antagonists/inverse agonists such as rimonabant, the clinical concerns that have emerged, and the possible future of cannabinoid-based therapy of obesity and related diseases.

2. The endocannabinoid system and cannabinoid-related compounds

The endocannabinoid system (ECS) is a lipid signaling system which includes the cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the enzymatic machinery for their synthesis and inactivation (Matias and Di Marzo, 2007). Endocannabinoids are important neuromodulators that appear to be involved in a plethora of physiological processes such as modulation of synaptic transmission, nociception, regulation of motor activity, cognitive processes, neuroprotection, immune function and inflammatory responses, antiproliferative actions in tumoral cells, control of cardiovascular system, and neurodevelopment, among others (Marsicano and Lutz, 2006). Notably, the ECS appears to be critically involved in the homeostasis of energy balance (Matias and Di Marzo, 2007).

Endocannabinoids are polyunsaturated fatty acid derivatives. An *N*-ethanolamide of arachidonic acid, anandamide (AEA), and a glyceryl ester of arachidonic acid, 2-arachidonoylglycerol (2-AG), are the most studied endocannabinoids. Paralleling AEA, there are a group of *N*-acylethanolamides (oleylethanolamide, palmitoylethanolamide, homo-gamma-linoleylethanolamide and docosatetraenylethanolamide) that arises from a common membrane phospholipid, sharing with AEA the enzymes for biosynthesis and degradation, though they do not target the cannabinoid receptors as a primary receptor (Matias et al., 2007). Although not considered extensively in the present review, it should be noted that some of these compounds may play roles as "entourage compounds" with the endocannabinoids to regulate homeostatic functions. The "entourage effect" relies on the co-release of other fatty acid derivatives that can potentiate endocannabinoids action, with the underlying mechanisms yet to be discovered.

The anabolic and catabolic pathways for AEA and 2-AG appear to rely on very complex enzymatic cascades that are in the progress of being elucidated. In brief, AEA can be generated from *N*-arachidonoylphosphatidylethanolamine via parallel biosynthetic pathways, whereas 2-AG can be generated by diacylglycerol lipase from diacylglycerol substrates and, alternatively, it can also be produced from triglycerides through a lipase-mediated enzymatic process (Di Marzo, 2008a). Due to their lipophilic nature, endocannabinoids cannot be stored in vesicles and, thus, they are considered "non-classical" neurotransmitters; they are synthesized and released "on demand" in response to diverse physiological and pathological stimuli (Piomelli, 2003). Endocannabinoid-inactivating mechanisms include cellular reuptake and hydrolysis. Though the putative reuptake transporter has not been isolated or cloned yet, there are compounds that are considered as inhibitors of cellular uptake. A fatty acid amide hydrolase is the main AEA hydrolase, whereas a monoacylglycerol lipase is critical in degrading 2-AG (Di Marzo, 2008a). Compounds that enhance endocannabinoid signaling by inhibiting endocannabinoid reuptake (e.g., AM404, VDM11, OMDM-1, OMDM-2, and UCM707) or by inhibiting degradation (e.g., the fatty acid amide hydrolase inhibitors URB597, AM374, or *N*-arachidonoyl-serotonin) are widely used in preclinical studies and may have potential therapeutic applications (Di Marzo, 2008a).

Cannabinoids mainly exert their pharmacological effects by the activation of specific membrane receptors. Mammalian tissues contain at least two types of cannabinoid receptors, CB_1 and CB_2, which are metabotropic receptors coupled to G-proteins of the Gi/o type. Transduction systems include inhibition of adenylyl cyclase and inhibition of certain voltage-sensitive calcium channels (predominantly, those found presynaptically), and activation of inwardly-rectifying potassium channels, mitogen-activated protein kinase, and focal adhesion kinase (Howlett et al., 2002). The presence of an allosteric site on the CB_1 receptor is an important finding since it opens new possibilities for the assay and possible therapeutic utility of CB_1 allosteric modulators, including regulation of body weight and food intake (Ross, 2007).

CB_1 receptors are expressed, among other areas, in the olfactory bulb, neocortex, pyriform cortex, hippocampus, amygdala, basal ganglia, thalamic nuclei, cerebellar cortex and brainstem nuclei (Piomelli, 2003). Although CB_1 receptor expression in the hypothalamus is relatively lower, the ECS nonetheless exerts important functions in this region (Marsicano and Lutz, 2006). According to this, the hypothalamic ECS appears to play a crucial regulatory role in modulating food intake and other aspects of energy metabolism (Cota and Woods, 2005). Regarding the peripheral distribution of CB_1, it is expressed in metabolically-relevant tissues, e.g. adipose tissue (Cota et al., 2003), liver (Osei-Hyiaman et al., 2005), skeletal muscle (Cavuoto et al., 2007) and the endocrine pancreas (Juan-Picó et al., 2006). Its contribution to the physiology of these organs is under ongoing investigation, although its activation seems to promote lipogenesis and energy storage.

CB_2 receptors are mostly peripherally located on immunological tissues, and therefore implicated in immunological functions (Howlett et al., 2002). However, they have also been found within the central nervous system. Immunohistochemical analyses have revealed CB_2 receptors in apparent neuronal and glial processes in diverse rat brain areas, including cerebellum and hippocampus (e.g. Suárez et al., 2008). These recent findings change the classical view of peripherally located CB_2 receptors and suggest broader functional roles for these receptors. In addition, CB_2 receptors have been also described in peripheral tissues involved in metabolism and energy homeostasis like adipose (Roche et al., 2006), skeletal muscle (Cavuoto et al., 2007) and endocrine pancreas (Juan-Picó et al., 2006). Interestingly, far beyond the expression of CB_2 in these tissues, its ability in modulating key metabolic processes, e.g. insulin secretion from pancreatic beta cells, has been reported (Juan-Picó et al., 2006).

It has been shown that some of the effects of AEA are mediated by the transient receptor potential vanilloid type-1 channel (TRPV1), formerly called vanilloid receptor VR1 (Howlett et al., 2002). These receptors have been traditionally known for their function in sensory nerves where they mediate perception of inflammatory and thermal pain, but they are also expressed within the brain contributing to other important physiological functions (Howlett et al., 2002). Evidence for co-expression of CB_1 and TRPV1 receptors in diverse brain regions has opened new avenues for the study of possible functional relationships between these receptor families. In fact, there are several similarities between CB_1 and TRPV1, in terms of opposing actions on the same intracellular signals, roles in the same pathological conditions, and shared ligands and tissue distributions (Starowicz et al., 2007). Interestingly, TRPV1 receptors are also expressed in rodent endocrine pancreas, stimulating insulin secretion, an opposite effect to that exerted by CB1 receptors (Akiba et al., 2004). Finally, an additional G-protein-coupled receptor, GPR55, has been proposed as a possible new cannabinoid receptor that might play a physiological role in lipid or vascular biology (Baker et al., 2006).

3. Tools for pharmacological manipulation of the endocannabinoid system

After the discovery of both the CB_1 receptor and AEA, an intense research effort has yielded numerous drugs that interact with most of the main elements of the ECS. Today we have drugs that bind to the CB_1 receptor as agonists or antagonists, drugs that block endocannabinoid transport (see preceding section), drugs that inhibit the activity of fatty acid amide hydrolase or monoacylglycerol lipase, and selective inhibitors for the enzymes diacylglycerol lipase α and β, which catalyse the synthesis of 2-AG (Di Marzo, 2008a). Although specific *N*-arachidonoylphosphatidylethanolamine-phospholipase D inhibitors are still lacking, it is reasonable to think that they will be available in a short time. As a summary of cannabinoid pharmacology, Table 1 shows the reference compound for each molecular target.

Cannabinoid receptor agonists may be designed to mimic the signaling processes mediated by AEA and 2-AG, mainly in pathological situations where a boost in cannabinoid receptor stimulation might be needed. For example, Δ^9-THC (dronabinol) and nabilone (a synthetic Δ^9-THC derivative) are being used as to as anti-emetics to ameliorate vomiting and nausea in cancer patients and as orexigenic factors to prevent cachexia in AIDS patients (reviewed in Pertwee, 2009). By contrast, cannabinoid receptor antagonism might be the approach selected in situations of pathologically-enhanced endocannabinoid signaling (reviewed in Di Marzo, 2008a). The majority of this review is focused on cannabinoid receptor antagonists because of their clinical use for the treatment of complicated obesity.

Several CB_1- and CB_2-selective antagonists have been developed. Antagonists include the CB_1-selective SR141716A (rimonabant), MK-0364 (taranabant), SR 147778 (surinabant), CP-945598, SLV-319, AM251, AM281 and LY320135, and the CB_2-selective SR144528 and AM630 (reviewed in Di Marzo, 2008a). These compounds all behave as inverse agonists, i.e. they exert the opposite pharmacological effect of a receptor agonist; Thus, they not only block the potential binding of an agonist, but they also abolish the constitutive activity of these receptors, i.e. the intrinsic activation without the action of a ligand upon them. There are less reports on 'neutral' cannabinoid receptor antagonists but they have also been developed (Ruiu et al., 2003; Pavon et al., 2006). Although cannabinoid antagonism is a solid pharmacological strategy for therapeutic development, to date only those with inverse agonist activity, and not neutral antagonists or partial antagonists, have reached clinical trials in humans.

Table 1
Targeting the endogenous cannabinoid system: synthetic drugs of reference for CB_1 and CB_2 receptors, anandamide transporter (AT) and the main synthesizing and degrading enzymes of endocannabinoids. Abbreviations: CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; AT, anandamide transporter, FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; DAGL, diacylglycerol lipase.

Name	Target	Action	Ki/IC50 (nM)	Reference
ACEA	CB_1	Agonist	1.4	J Pharmacol Exp Ther 1999;289:1427
SR141716A	CB_1	Antagonist/inverse agonist	5.6	FEBS Lett 1994;350:240
HU-308	CB_2	Agonist	22.7	Proc Natl Acad Sci U S A 1999;96:14228
SR 144528	CB_2	Antagonist	0.60	J Pharmacol Exp Ther 1998;284:644
AM404	AT	Blocker	800	Science. 1997;277:1094
OL-135	FAAH	Inhibitor (reversible)	2.1	J Pharmacol Exp Ther 2004;311:441
URB597	FAAH	Inhibitor (irreversible)	4.6	Nat Med 2003;9:76
URB602	MAGL	Inhibitor (reversible)	15 μM	Br J Pharmacol 2007;150:186
O-5596	DAGL	Inhibitor	100	ChemMedChem. 2009;4:946

4. Role of the endocannabinoid system in the regulation of energy metabolism: an overview

In the early 1960s Δ^9-tetrahydrocannabinol (Δ^9-THC) was identified as the primary active ingredient responsible for the psychotropic effects of marijuana (Gaoni and Mechoulam, 1964). Although the appetite-inducing properties of cannabis have been known for centuries, it was not until recently that orexigenic properties of its main psychoactive compound, Δ^9-THC, were clearly demonstrated (Williams et al., 1998). Increasingly during the last years, a substantial body of evidence has accumulated implicating the ECS in the regulation of appetite, eating behavior and body weight at both central and peripheral sites (Matias and Di Marzo, 2007). Moreover, it has been proposed that the principal functional role of endocannabinoids through actions at CB_1-receptors is reorienting energy balance towards energy storage, which in humans principally means increasing lipid production and accumulation (Piazza et al., 2007).

The ECS is strategically positioned to influence virtually every key point of the regulatory network that controls energy homeostasis (Cota and Woods, 2005). In the brain the ECS appears to control food intake mainly at two functional levels, i.e. the hypothalamus and the limbic system. The hypothalamic ECS modulates feeding by decreasing satiety signals and enhancing orexigenic signals. In addition, through interactions with the mesolimbic pathways involved in reward mechanisms, endocannabinoids appear to increase eating motivation, possibly reinforcing the incentive or hedonic value of food (reviewed in Kirkham, 2003). To discriminate between food intake (free-food intake) and food reinforcement (motivation for food), the progressive ratio schedule of reinforcement methodology has been used. Studies using this methodology have revealed that Δ^9-THC increases the effort that an animal will exert to obtain food while the CB_1 antagonist/inverse agonist rimonabant reduces this effort (Solinas and Goldberg, 2005; Rasmussen and Huskinson, 2008). These studies provide a clearer characterization that food reinforcement may be a key behavioral mechanism altered by cannabinoid drugs (see also below).

The hypothalamus is the key brain structure that integrates peripheral satiety and adiposity signals that control eating behavior (Cota and Woods, 2005). Although CB_1 receptors are expressed at relatively low levels in the hypothalamus, their activation in this region nonetheless produces profound effects (Breivogel and Childers, 1998). CB_1 mRNA has been colocalized with the hypothalamic neuropeptides corticotropin-releasing hormone, cocaine-amphetamine regulated transcript, pre-pro-orexin and melanin concentrating hormone (Cota et al., 2003) suggesting that endocannabinoids influence signaling mediated by these neuropeptides in hypothalamic circuits controlling appetite and energy expenditure. Interestingly, AEA, when infused into the hypothalamic ventromedial nucleus, is able to promote food intake through CB_1 activation, and intrahypothalamic pretreatment with rimonabant attenuates this effect of AEA (Jamshidi and Taylor, 2001). Taken together, these and related results support a substantial role of the hypothalamic ECS in modulating food-related signaling in this region.

The hypothalamic paraventricular nucleus could also be implicated in integrating stress-related signals that influence food intake. At this level, rapid glucocorticoid actions mediated by a membrane glucocorticoid receptor may provide an integrative signal linking stress with the regulation of energy and fluid homeostasis. According to the model proposed by Tasker (2006), glucocorticoids would inhibit paraventricular nucleus and supraoptic nucleus neurons by stimulating a rapid synthesis and release of endocannabinoids. The endocannabinoids would be transmitted retrograde to presynaptic glutamate terminals where they suppress glutamate release (synaptic excitation) through presynaptic CB_1 receptor activation (Tasker, 2006).

The limbic system constitutes another functional level of control over food intake. Prefrontal cortex, amygdala, the ventral tegmental area, the nucleus accumbens and the hippocampus, among others, are

discrete cortico-limbic structures that influence motivated behaviors. The natural rewarding properties of food are well known, mediated primarily by dopamine release in the mesolimbic pathway (reviewed in Wise, 2004). Growing evidence implicates an interaction between mesolimbic endocannabinoid and dopamine systems in regulation of food intake (reviewed in Di Marzo et al., 2009). For example, dopamine inhibits endocannabinoid biosynthesis in the limbic forebrain (Patel et al., 2003), administration of 2-AG into the shell of the nucleus accumbens produces hyperphagia (Kirkham et al., 2002) and AEA, through activation of the mesolimbic dopaminergic system, participates in the signaling of brain reward processes (Solinas et al., 2006). The interactions of cannabinoids and dopamine in the nucleus accumbens remain to be clearly elucidated. Reward mediated by food or food memories can be modulated by endocannabinoid-dopamine interactions at the level of the intrinsic projecting neurons of the nucleus accumbens. Although accumbens-projecting dopamine neurons are devoid of cannabinoid receptors, and there is no co-localization of dopamine and CB_1 receptors in the shell of the accumbens, the ECS is present in cortical (i.e. prefrontal) and subcortical (i.e. amygdalar) glutamatergic/gabaergic afferents that are regulated by dopamine input (Julian et al., 2003).

A putative mechanism by which endocannabinoids modulate motivation to consume food seems to be by increasing the palatability associated with the reward of food (Kirkham, 2003). Thus, some studies have suggested that peripheral administration of rimonabant specifically reduced intake of palatable foods (Gallate and McGregor, 1999) whereas Δ^9-THC has been reported to increase the preference for palatable food (Brown et al., 1977) and rats exposed to a palatable diet for 10 weeks displayed downregulated CB_1 mRNA in several limbic areas (Harrold et al., 2002). However other authors have found that rimonabant also decreased the intake of normal foods, suggesting that high palatability is not necessary to obtain a rimonabant-mediated anorectic effect (Freedland et al., 2000). A reconciliatory view could be related to the intermediacy of different populations of CB_1 receptors in the effects of exogenous and endogenous CB_1 agonists. Thus, while systemic CB_1 agonists might preferentially enhance incentive properties of food, the CB_1 receptors tonically activated by endocannabinoids (whose activity is revealed by the use of systemic CB_1 antagonists/inverse agonists) may be involved in both the homeostatic and hedonic aspects of food intake (Di Marzo et al., 2009).

The ECS not only modulates food intake but also energy expenditure. Experiments using the pair-feeding paradigm (in which experimental and control animals consume the same amount of food; normally used to distinguish between food intake-dependent and independent effects) have shown that CB_1 knockout mice have increased energy expenditure (Cota et al., 2003) and they are resistant to diet-induced obesity despite their overall caloric intake being the same as wild type littermates (Di Marzo et al., 2001). Furthermore, diet-induced obese mice treated with rimonabant showed a transitory reduction in food intake but a sustained reduction in body weight, suggesting increased energy expenditure (Ravinet Trillou et al., 2003). This finding was supported by another study recently (Osei-Hyiaman et al., 2008).

Studies with CB_1 knockout mice have also supported a role of the ECS in the regulation of appetite and lipid metabolism. When maintained on standard chow diet, CB_1 knockout mice were slightly hypophagic, and their body weight and adiposity were lower than that of the wild-type mice (Cota et al., 2003). After introduction of a high-fat diet, the CB_1 knockout animals did not display hyperphagia, did not become obese and did not develop the insulin resistance normally occurring under this type of diet (Ravinet Trillou et al., 2004). Studies with CB_1 knockout mice also offered early evidence regarding potential adverse effects of reduced CB_1 receptor signaling: mice lacking CB_1 showed anxiogenic-like behavior, depressive-like behavior, anhedonia, greater aggression, and suffered higher rates of epilepsy, age-related neuron loss, and premature mortality (Zimmer et al., 1999).

In addition to the role of the ECS within the brain, the peripheral ECS also regulates energy balance, in particular by peripheral lipogenic mechanisms and modulation of lipid and carbohydrate metabolism. This has been demonstrated by chronic treatment with rimonabant that not only resulted in reduction of body weight gain, but also in significant improvement in lipid profiles (reduced triglycerides and cholesterol), and glucose tolerance in obese humans and rodents (Bensaid et al., 2003; Scheen et al., 2006). Evidence suggests that the activation of CB_1 receptors in these peripheral tissues promotes lipogenesis, lipid storage, insulin secretion, glucagon secretion and adiponectin modulation (Cota et al., 2003; Osei-Hyiaman et al., 2005; Bermúdez-Silva et al., 2008). These findings confirm a prominent role for peripheral CB_1 receptors on the modulation of metabolism.

Taken together, these findings indicate that both central and peripheral CB_1 receptors act in a coordinated fashion to regulate energy homeostasis. A potential link between central and peripheral signals may focus on leptin, whose signaling is dependent on an intact brain ECS (Di Marzo et al., 2001), although insulin constitutes also an important link between peripheral tissues and the hypothalamus (Xue and Kahn, 2006). These hormonal signals together with diet nutrients, e.g. glucose, long-chain fatty acids and branched-chain amino acids, are integrated in the hypothalamus, at least in part through the mammalian target of rapamycin complex 1 and AMP-activated protein kinase signaling, to modulate food intake (Cota, 2009).

5. CB_1 receptor blockade as therapeutical strategy to fight against obesity

Obesity is defined as excessive adiposity, and it is diagnosed based on body mass index (BMI), weight in kilograms per square of height in meters. People with BMI between 25 and 29 are diagnosed as overweight and those with BMI greater than 30 as obese. Excessive adiposity leads to increased risk of developing pathologies like type 2 diabetes and coronary heart disease. Obesity seems to be a condition associated with a pathological overactivation of the ECS (Matias and Di Marzo, 2007); therefore, restoring a normal endocannabinoid tone by drugs that interfere with the ECS could theoretically help to arrest both the development and the maintenance of obesity and obesity-related co-morbidities (Berry and Mechoulam, 2002). In fact, as discussed above CB_1 receptor antagonists/inverse agonists such as rimonabant (SR141716A) have been shown to reduce food intake, reduce motivation for food and improve metabolic parameters in animal models of obesity (Colombo et al., 1998; Solinas and Goldberg, 2005; Rasmussen and Huskinson, 2008; Ravinet Trillou et al., 2003). Interestingly, while food intake inhibition was only transitory, suggesting the development of CNS tolerance, improved metabolic parameters and weight loss were maintained over time. The main conclusion arising from experimental data in humans and animal models is that endocannabinoids seem to be engaged in the coordination of energy homeostasis at multiple sites, central and peripheral, and that a dysregulation of this homeostatic function is present in obesity conditions, with or without additional complications such as diabetes and dyslipidemia. This suggests that pharmacological manipulation of the ECS could be an appropriate therapeutical strategy for obesity management.

On the basis of the experimental evidence (inhibition of food intake, induction of weight loss and improvement of metabolic parameters), the CB_1 antagonist/inverse agonist rimonabant has been assessed for the treatment of obesity and metabolic disorders (metabolic syndrome) in four published clinical trials: RIO-(*R*imonabant *I*n *O*besity)-Europe (Van Gaal et al., 2005), RIO-Lipids (Després et al., 2005), RIO-North America (Pi-Sunyer et al., 2006), and RIO-Diabetes (Scheen et al., 2006). Administration of the drug has been associated with weight reduction (although a regain of weight was observed after discontinuation of the treatment), an increase in high-density lipoprotein cholesterol, a decrease in triglyceride concentrations, reductions in plasma glucose

and insulin levels, decreased plasma leptin levels and increased plasma adiponectin levels. In addition to rimonabant, several other CB_1 receptor antagonists/inverse agonists are in the drug development pipeline (e.g. taranabant, CP-945598, SLV-319).

Based on these clinical trials data, on 21 June 2006, the EMEA approved the sale of rimonabant (Acomplia®) in the European Union. The EMEA approved Acomplia® in combination with diet and exercise for the treatment of obese patients and overweight patients with BMI>27 kg/m^2 and associated risk factors such as dyslipidemia or type 2 diabetes. Rimonabant was released in Argentina, Austria, Brazil, Denmark, Finland, Germany, Greece, Ireland, Mexico, Norway, Sweden, Switzerland, and the United Kingdom.

6. Clinical concerns regarding systemic CB_1 blockade as a treatment for obesity

As they were published, the RIO trial publications were sometimes accompanied by probing editorials (e.g., Cleland and Sattar, 2006), and were followed by nearly a dozen critical commentaries (e.g., Roberfroid, 2007). Criticisms included the use of unvalidated or disputed surrogate endpoints, favorable claims not supported by trial data, overstated treatment efficacy, downplayed adverse effects, lack of internal validity and external validity or generalizability, and failure to disclose financially-conflicted interests.

Five meta-analyses of rimonabant clinical trials highlighted safety concerns (Curioni and Andre, 2006; Christensen et al., 2007; Rucker et al., 2007; Chavez-Tapia et al., 2009; Johansson et al., 2009). In contrast, one meta-analysis concluded that rimonabant was safe and effective, but it was conducted by the same people who conducted the RIO trials (Van Gaal et al., 2008). Industry-funded meta-analyses tend to be less transparent, have more methodological flaws, and make more pro-industry conclusions regarding drugs than do independent meta-analyses (Jorgensen et al., 2006).

While seeking FDA approval in the face of these published concerns, the company that published the RIO trials submitted their raw data to the FDA. The FDA noted that certain adverse events in subjects receiving rimonabant went unreported in RIO publications, including 7 likely seizures, 26 cases of suicidality (suicidal ideations, suicide preparations, and suicide attempts), and one death in a rimonabant-treated subject ruled a suicide by the FDA (U.S. Food and Drug Administration, 2007). The FDA also questioned the generalizability of the RIO trials. Individuals with a history of anti-depressant medication were excluded from RIO studies, and subjects that required treatment with anti-depressants were discontinued from the studies. In real clinical practice, the FDA estimated that 30% of patients on weight-loss drugs received concurrent prescriptions for anti-depressant medication.

The FDA proposed a physiological rationale for adverse events seen with rimonabant: The ECS modulates physiological responses to repetitive stress conditions and in pathological conditions, such as anxiety, phobias, depression, and posttraumatic stress disorders — the endocannabinoid system has an 'autoprotective' role (U.S. Food and Drug Administration, 2007). Thus, while being used against obesity, rimonabant might interfere with endocannabinoid-mediated adaptation to new stressful conditions, thus explaining the anxiogenic and pro-depressant effects observed in obese patients treated with this drug (Di Marzo, 2008a). Moreover, critical components of the reward system that plays an important role in naturally motivated behaviors like feeding, sex, or social interactions express CB_1 receptors (Melis et al., 2007). Consequently, it is not surprising that rimonabant also blocks pathways for natural rewards other than food, which contributes to mood disorders and depression. In addition, rimonabant might cross-react with other drugs that augment the ECS system, such as tricyclic anti-depressants, diazepam, paracetamol (acetaminophen), nonsteroidal anti-inflammatory drugs, and docosahexaenoic acid (fish oil) supplements, as well as clinical efforts to elevate endocannabinoid tone, such as aerobic exercise, spinal manipulation, massage, and perhaps acupuncture (reviewed in McPartland, 2008).

On 13 June 2007 the FDA voted against approval for rimonabant, in part, because they found 26% of subjects who took rimonabant in clinical studies suffered depressed mood, irritability, agitation, anxiety, insomnia, headache, or other adverse psychiatric events in comparison to 14% people suffering these symptoms in the placebo group (U.S. Food and Drug Administration, 2007). On 19 July 2007 the EMEA modified the indications for rimonabant, stating that patients with a history of psychiatric (mostly affective) disorders should not receive the drug. The EMEA continued to monitor post-marketing safety data. On 23 October 2008 the EMEA recommended that sales of rimonabant be suspended, after noting five rimonabant-associated suicides between June and August of that year (European Medicines Agency, 2008). Shortly after that decision, Sanofi-Aventis, Merck, Pfizer, and Solvay announced that they would stop further clinical research on cannabinoid receptor antagonists/inverse agonists. The EMEA officially withdrew approval of rimonabant on 16 January 2009.

More recently, however, the company that conducted the RIO trials also tested the efficacy of rimonabant to decrease coronary artery atherosclerosis (Nissen et al., 2008), to improve glycemic control (Rosenstock et al., 2008), and to reduce the accumulation of intra-abdominal fat and liver fat (Després et al., 2009). Rimonabant plus a 600-kcal/day caloric deficit diet improved glycemic control and reduced HbA(1c) 0.51% compared to placebo (Rosenstock et al., 2008). Rimonabant compared to placebo reduced intra-abdominal fat and liver fat by −10.1% and −11.5%, respectively (Després et al., 2009). The results from these studies suggested that careful patient selection could improve the risk:benefit ratio afforded by rimonabant. For example, patients with no history of depression could be treated more safely than people with a history of depression (Di Marzo, 2008b).

Although the risk–benefit ratio of cannabinoid receptor blockade with antagonist/inverse agonist compounds may preclude its use for *chronic* conditions such as obesity, cannabinoid receptor blockade could serve in the treatment of *acute* endocannabinoid dysregulation, such as hepatic cirrhosis, hemorrhagic or endotoxic shock, cardiac reperfusion injury, and doxorubicin-induced cardiotoxicity (McPartland, 2008). Proponents of rimonabant believe that the "regulatory bar for the approval of anti-obesity drugs has been raised" (Heal et al., 2009). The FDA has been perceived by some as over-vigilant with rimonabant, perhaps because of the recent imbroglio surrounding Vioxx (rofecoxib).

The fact remains that none of the pharmaceutical treatments for obesity developed to date have been fully effective: they lose efficacy after a period of 6–12 months into treatment, need supportive lifestyle adjustments (nutritional changes and physical activity) to observe clear-cut benefits, and usually a regain of lost weight is observed after treatment is completed or discontinued. The reasons underlying these therapeutic failures may be related to the development of compensatory mechanisms that override the pharmacological action of the drug and the inability in returning to a previous "healthy" steady-state in energy balance. Regarding this latter issue, obesity could be considered the consequence of an allostatic change that leads to a "pathological" steady-state with increased risk of developing type 2 diabetes and related cardiovascular diseases (McEwen and Wingfield, 2003). Thus, the most suitable treatment should be able to reverse the allostatic change. It is reasonable to think that a therapeutic adjunctive strategy (see next section) could be the best option to reverse the allostatic change. In the next section we provide information on several alternatives to systemic CB_1 antagonists/inverse agonists that could help in the pharmacological treatment of obesity, including the combined use of several anti-obesity drugs.

7. Alternatives to systemic CB_1 antagonists/inverse agonists

Rimonabant is a CB_1 antagonist with full inverse agonist activity and high binding affinity at CB_1. Its use as therapeutic tool has been

controversial and it is likely that most pharmaceutical companies will be deterred from developing a drug displaying rimonabant-like CB_1 receptor inverse agonist/antagonist activity. However, drugs that modulate ECS function should not be uniformly stigmatized based on the findings with the class of compounds possessing inverse agonist activity. The ECS is increasingly recognized to maintain homeostasis in health and disease (Pertwee, 2005) and it is expressed in all key points regarding energy balance. In this context, there is an urgent need for a new strategy for blocking CB_1 receptors that shares the effectiveness of rimonabant against obesity, type 2 diabetes and related cardiometabolic risk factors but not its apparent ability to induce anxiety and depression/suicidal ideation in some patients (Pertwee, 2009).

These undesirable effects of rimonabant may be related to blocking constitutive CB_1 activity. Perhaps the use of neutral antagonists, devoid of this effect, could maintain weight loss while avoiding unwanted side effects (Greasley and Clapham, 2006). Examples of this kind of drugs may be the recently described LH-21, derived from a 1,2,4-triazole (Pavon et al., 2006), AM4113, a pyrazole analog structurally related to SR141716 and AM251 (Sink et al., 2008), or NESS 0327, a rimonabant analog (Ruiu et al., 2003). These drugs have been reported not to induce nausea in rats and ferrets (Salamone et al., 2007) and displayed anorectic properties (Pavon et al., 2006; Salamone et al., 2007).

Taking into consideration that many of the undesired side effects are psychiatric disturbances relying upon central nervous system action, an additional alternative may lie on the development of peripherally-restricted antagonists unable to cross the blood–brain barrier. An example of this kind of drug is the recently described LH-21 that has a poor CNS penetration. LH-21 was able to induce weight loss in rats (Pavon et al., 2006) but, however, it failed in improving metabolic parameters in Wistar and Zucker rats, suggesting that lack of inverse agonism, or poor penetration in the brain, might limit the metabolic benefits of CB_1 receptor blockade (Pavón et al., 2008). Recently, a new compound, URB447, has been identified as a mixed CB_1 antagonist/CB_2 agonist with anorectic actions and devoid of central nervous system effects (LoVerme et al., 2009).

Another alternative is the use of partial agonists of CB_1 receptors. These molecules do not impair constitutive activity at CB_1 receptors, suggesting that they will be devoid of at least some of the inverse agonist-related side effects reported; thus, partial agonists could prevent psychiatric side effects while simultaneously serving as partial antagonists by blocking the binding of extra obesity-induced endocannabinoids with full agonist efficacy at CB_1 receptors. Partial agonists serve well as partial antagonists when levels of endogenous agonists are elevated (the exact scenario seen with endocannabinoids and obesity; Matias and Di Marzo, 2007). Partial agonists have been found useful in cardiology (e.g., pindolol), psychiatry (e.g., aripiprazole, buspirone), and substance abuse treatment (e.g., varenicline, buspirone), with fewer adverse affects than antagonists or inverse agonists, and without compromising clinical efficacy (Ohlsen and Pilowsky, 2005). They may reverse obesity and metabolic syndrome, including the 5-HT_6 ligand E-6837 (Fisas et al., 2006), and a Chinese herbal formula with an undisclosed herb that acts at CB_1 (Qiu, 2007). Finally, partial agonists may act as pleiotropic drugs, also known as "selectively non-selective drugs." These "magic shotguns" interact with several molecular targets, and can provide a superior therapeutic effects and side effect profile compared to the action of a selective, single "magic bullet" (McPartland and Pruitt, 1999).

Pharmacological modulation of endocannabinoid levels, rather than CB_1 blockade, may provide a more physiological approach to treating obesity. Excessive endocannabinoid levels, rather than excessive CB_1 expression, appear to be the primary defect associated with obesity (Matias and Di Marzo, 2007). In fact, it has been reported an up-regulation of CB_1 receptors in the adipose tissue of obese rats (Bensaid et al., 2003), but a down-regulation in certain extrahypothalamic regions (Harrold et al., 2002), suggesting tissue-specific changes in obese animals. However, the ECS seems to be overactive in obesity and blockade of CB_1 may induce a feedback loop that drives endocannabinoid levels higher, and endocannabinoids may begin targeting other receptors, such as CB_2, TRPV1, several peroxisome proliferator-activated receptor paralogs or even the orphan G-protein-coupled receptor, GPR55. Effects from these crossover activations cannot be predicted. Thus, drugs targeting enzymes responsible for synthesis and degradation of endocannabinoids could be useful in counteracting the ECS dysregulation reported in obesity. An example of this compound could be the recently described O-5596 (Bisogno et al., 2009), a diacylglycerol lipase inhibitor that decreases endocannabinoid synthesis. Interestingly, this drug recently has been reported to decrease food intake in mice (Bisogno et al., 2009).

Another putative strategy is related to allosterism. The CB_1 receptor contains allosteric-binding sites that modulate the regular (orthosteric) ligand binding site (Ross, 2007). Allosterism offer advantages over direct CB_1 blockade and endocannabinoid enzyme inhibition. Allosteric modulation only occurs in the presence of endogenous agonists, thereby resulting in a selective "fine-tuning" of the receptor when and where it is needed. PSNCBAM-1, a novel allosteric antagonist, inhibited appetite and produced weight loss in rats (Horswill et al., 2007). Finally, an adjunctive strategy that exploits synergism of blocking CB_1 and other anti-obesity drugs could also be a valid approach (e.g. a combination of a low dose of a CB_1 receptor antagonist and some other type of anti-obesity agent; Pertwee, 2009).

8. Conclusions

The ECS plays a critical role in the regulation of food intake and energy metabolism. Interestingly, it is positioned throughout the body in virtually all key points modulating feeding, making it an attractive target for treating obesity and related diseases. Clinical trials with the CB_1 antagonists/inverse agonists rimonabant and taranabant have verified proof-of-concept by demonstrating efficacy at reducing food intake as well as obesity and metabolic alterations. However, antagonists/inverse agonists have also induced serious adverse effects, suggesting they are not the best option for an ECS-based therapy of obesity and related diseases. Future directions should be focused to:

1) further increase our knowledge regarding ECS physiology, including a more precise understanding of the role of the peripheral ECS and a better elucidation of ECS genetic dysfunctions that give rise to disease-related phenotypes. A crucial point will be to focus research on where and how both peripheral and central signals are integrated leading to a single behavioral response.
2) test the efficacy of other ECS-based potential treatments, including as described above the use of partial agonists, neutral antagonists, peripherally-restricted antagonists, allosteric modulators, modulators of ECS enzymes, and adjunctive strategies.

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