Title: The role of the endocannabinoid system in eating disorders. Pharmacological implications

Running title: Endocannabinoid system in eating disorders

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Conflicts of interest and source of funding: none declared.

Abstract

The endocannabinoid (eCB) system is a widespread intercellular signalling mechanism that plays a critical role in body homeostasis. It is located in key points involved in food intake and energy expenditure, coordinating all the players involved in energy balance. As such it has arisen as an interesting target for the management of diseases characterized by an imbalanced energy homeostasis, like obesity and eating disorders. The aetiology of eating disorders and the molecular systems involved are still largely a mystery. Research has focused on brain circuits where the eCB system plays an important role, like those related to feeding behaviour and the rewarding properties of food. Accordingly, recent findings have suggested a deregulation of the eCB system in eating disorders. At present, cannabinoid agonists are safe and effective tools in the management of diseases in which weight gain is needed, e.g. cachexia in AIDS patients. However, studies on the potential therapeutic validity of cannabinoids in eating disorders are scarce and inconclusive. Taken together, all these considerations warrant more preclinical and clinical investigations in the role of the eCB system in eating disorders. Eventually, they may provide novel pharmacological approaches for the treatment of these diseases.

Keywords:

CB1R, endocannabinoids, anandamide, anorexia nervosa, bulimia nervosa, endocannabinoid-based therapy

A brief update on the endocannabinoid (eCB) system

Derivatives from *Cannabis sativa*, such as marijuana and hashish, as well as delta-9-tetrahydrocannabinol (THC), the major psychoactive component of the plant, have long been known to stimulate appetite (Foltin et al., 1986). Similarly, oral THC has been reported to produce hyperphagia in satiated rats (Williams and Kirkham, 2002). The identification of specific binding sites for THC in the central nervous system (CNS) allowed the identification of an endogenous eCB system and provided the first clues for understanding the molecular mechanisms involved in the influence of cannabinoids in eating behaviours. The endogenous cannabinoid ligands, called endocannabinoids are polyunsaturated fatty acid derivatives that act through the activation of G-protein-coupled cannabinoid receptors, CB type 1 receptor, CB1R (Herkenham et al., 1991) and type 2 receptor, CB2R (Munro et al., 1993). Despite our knowledge of the molecular architecture of the eCB system have rapidly expanded in the last decades, the physiology, concentration and regionalized distribution of its components remain largely elusive.

Endocannabinoids, due to their lipophilic nature, are synthesized and released 'on demand' by the cleavage of membrane phospholipid precursors. Most widely studied endocannabinoids are N-arachidonoyl-ethanolamide (AEA) also called anandamide (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995, Sugiura et al., 1995), although other putative brain-derived lipids active at CB1Rs have been described (Keimpema et al., 2011). AEA concentrations are regulated by the conversion of a minor phosphoglyceride, N-arachidonyl-phosphatidylethanolamine (N-arachPE), via either a phospholipase D (NAPE-PLD) (Okamoto et al., 2009) or a two enzyme pathway that involves and alpha, beta hydrolase (ABH4) and a glycerophosphodiesterase (GDE1) (Simon and Cravatt, 2008). Similarly,

2AG is synthesized via a two-enzyme cascade of phospholipase C (PLC) and diacylglycerol lipase (DGL) (Bisogno et al., 2003). Ca^{2+}-dependent enzymes control biosynthesis pathways for both AEA and 2-AG release, although the mechanisms that regulate the activities of these enzymes are currently not well understood. As a whole, it has been suggested that the cascade of endocannabinoid production might be triggered after an increase in intracellular Ca^{2+} concentrations, usually in response to an acute or chronic perturbation of cell homeostasis and with the function of returning the cell to its steady state before the perturbation (Di Marzo, 2008). On the other side, regarding their deactivation, it is though that endocannabinoids can passively diffuse through lipid membranes, although a high affinity transporter -not yet identified- could accelerate this process. At least three enzymes are involved in the catabolism of AEA: fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), a lysosome-localized fatty acyl amide hydrolase with an acid optimum pH (NAAA) (Tsuboi et al., 2005) and a recently identified FAAH-2 localized in lipid droplets (Kaczocha et al., 2010). In contrast, brain 2-AG inactivation is mainly afforded by the enzyme monoacylglycerol lipase (MGL), with limited contributions by αβ-hydrolase (ABHD) 6 and 12 (Blankman et al., 2007). AEA and 2-AG-are also substrates for some arachidonate oxygenases, including lipoxygenases (Edgemond et al., 1998) and COX-2 (Kozak et al., 2002).

AEA and 2-AG behave as cannabinoid receptors agonists. AEA is also able to activate the transient receptor potential vanilloid type 1 (TRPV1) ion channel (Starowicz et al., 2007). Thus, AEA as an '*endovanilloid*' has been proposed as a second messenger that regulates calcium signalling through this receptor (De Petrocellis and Di Marzo, 2009). CB1Rs are expressed ubiquitously throughout the brain; they are found at highest concentrations in the hippocampus, neocortex, basal ganglia and cerebellum, while a moderate presence is observed in the basolateral amygdala,

hypothalamus, and midbrain [for review consult (Mackie, 2005)]. CB2Rs, initially proposed to be restricted to the periphery and associated to the immune system (Munro et al., 1993, Galiegue et al., 1995), has eventually been described in a diversity of locations within the body, such as muscle, liver, intestine, pancreas, testis and adipose tissue (Roche et al., 2006, Liu et al., 2009), and more recently in the brain. Human and animal studies have described the presence of CB2Rs in several regions of the CNS, including cerebral cortex, striatum, hippocampus, amygdala, periaqueductal grey (PAG), cerebellum and several hypothalamic nuclei under normal physiological conditions (Van Sickle et al., 2005, Gong et al., 2006, Onaivi, 2006, Suarez et al., 2008). Although CB1Rs and CB2Rs are well known and characterized, numerous pharmacological studies have suggested the existence of additional cannabinoid receptors [see (De Petrocellis and Di Marzo, 2010) for review]. Two G protein-coupled receptors, GPR55 and GPR119, have been proposed as novel potential cannabinoid receptors (Baker et al., 2006) though currently this is a controversial issue. Moreover, increasing evidence now suggests that endocannabinoids are also natural activators of the peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors (O'Sullivan and Kendall, 2010).

In general, endocannabinoids function as retrograde messengers to modulate the plasticity of many synapses in the adult brain (Regehr et al., 2009). It is widely accepted that endogenous activation of CBRs is regulated by processes that govern the biosynthesis and catabolism of endocannabinoids. Actually, recent evidence suggests that in neurons, the rate limiting step for the synthesis of 2-AG and cannabinoid actions is DGL (Yoshida et al., 2006, Gao et al., 2010). Available data indicate that 2-AG is produced by post-synaptic neurons in response to metabotropic receptor activation and/or depolarization, and targets CB1Rs present on pre-synaptic terminals (Pan et al.,

2009). Thus, 2-AG mediates activity-dependent retrograde inhibition of synaptic activity in many brain regions (Patel and Hillard, 2009), whereas AEA seems not to be involved in this process despite existing discrepant literature (Azad et al., 2004). Since extracellular AEA levels are lower than those of 2-AG (Caille et al., 2007), AEA has been suggested to provide low-intensity tonic activation of CB1R signalling, while 2-AG may function as a phasic high-intensity signal (Vaughn et al., 2010).

In the recent years, the eCB system has arisen as a widespread modulatory system involved in the homeostatic control of a plethora of physiological functions, including food intake and energy expenditure. It is thought to be usually silent and to become transiently activated after stressful conditions. Accordingly, increasing evidence supports the view that the activation of the eCB system plays important regulatory roles in the control of energy balance through central and peripheral mechanisms at multiple levels including the brain, gastrointestinal tract, liver, pancreas, muscle and adipose tissue [see (de Kloet and Woods, 2009, Andre and Gonthier, 2010, Maccarrone et al., 2010) for updated reviews].

The endocannabinoid system in energy homeostasis

The eCB system is strategically located in all the key points involved in food intake and energy expenditure, both at the central and the peripheral level. Thus, it is perhaps one of the few that can coordinate all the players involved in energy balance [reviewed in (Pagotto et al., 2006, Matias and Di Marzo, 2007)]. Together with its action on peripheral tissues, the eCB system influences feeding behaviour at the CNS by acting on circuits located in the hypothalamus, the reward system and the brain stem, with the overall net effect being anabolic [reviewed in (de Kloet and Woods, 2009, Di Marzo et al., 2009, Andre and Gonthier, 2010, Maccarrone et al., 2010)].

Briefly, the hypothalamus is a key brain structure involved in energy balance regulation. Despite the low expression of CB1R in the hypothalamus, a number of studies demonstrate that endocannabinoids through CB1Rs exerts a profound influence on the hypothalamic regulation of food intake [reviewed in (Bermudez-Silva et al., 2012)]. Also important is the role of CB1R in the hypothalamic leptin-mediated anorectic effects (Di Marzo et al., 2001). Leptin inhibits endocannabinoid production in the hypothalamus and, conversely, hypothalamic endocannabinoids are increased in genetically obese rodents lacking leptin or its receptor (Di Marzo et al., 2001). The reward system is a group of brain structures which regulate and control behaviour by inducing pleasurable effects. The major rewarding pathway in the brain is the mesolimbic pathway that goes from the ventral tegmental area via the medial forebrain bundle to nucleus accumbens, which is the primary release site for the main brain's pleasure chemical, i.e. the neurotransmitter dopamine. Cannabinoid CB1Rs are expressed in presynaptic glutamatergic and GABAergic nerve terminals in the ventral tegmental area, and endocannabinoids are synthesized by ventral tegmental area dopamine neurons, having a role in the fine-tuned regulation of these cells (Maldonado et al., 2006). While it is still unclear exactly what cell populations express CB1Rs in the nucleus accumbens, it seems that endocannabinoids within this area are able of increasing food intake in a CB1-dependent manner (Kirkham et al., 2002). Additional studies have also reported that endocannabinoids acting in the nucleus accumbens modulate the palatability of food (Mahler et al., 2007). The brainstem is also a relevant player in food intake regulation: satiety signals from the stomach and duodenum reach the brainstem through sensory and vagal fibres. Among these, cholecystokinin (CCK) and peptide YY have been related with the eCB system [reviewed in (Di Marzo et al., 2009, Bermudez-Silva et al., 2010)]. Cannabinoid CB1Rs are expressed in the

brainstem and in vagal afferent neurons modulating these signals (Burdyga et al., 2004, DiPatrizio and Simansky, 2008). Furthermore, endocannabinoid tone changes in the brainstem during the different phases of eating [reviewed in (Di Marzo et al., 2009)].

Pathophysiology of eating disorders

Food intake or eating is the process by which edible substances are consumed in order to balance the energy expenditure in living creatures. This process relies in physiologic mechanisms regulating appetite and the natural drive to eat. In some conditions human feeding behaviour is altered leading to diseases, collectively known as eating disorders. These are a group of disorders characterized by physiological and psychological disturbances in appetite or food intake. They can be divided into three main pathologies, i.e. binge-eating disorder (BED), Bulimia Nervosa (BN) and Anorexia Nervosa (AN). BED is associated with three or more of the following: eating until feeling uncomfortably full; eating large amounts of food when not physically hungry; eating much more rapidly than normal; eating alone due to embarrassment; feeling of disgust, depression, or guilt after overeating. Criteria includes occurrence on average, at least 2 days a week for 6 months. The binge eating is not associated with compensatory behaviour (i.e. purging, excessive exercise, etc.) and does not co-occur exclusively with BN or AN (from DSM-IV, 1994). BN is characterized by a cycle of binge eating followed by purging to avert weight gain. Purging methods often include self-induced vomiting, use of laxatives or diuretics, excessive exercise, and fasting. AN is characterized by the loss of appetite and is associated with other features including an excessive fear of becoming overweight, body image disturbances, significant weight loss, refusal to maintain minimal normal weight, excessive exercise and amenorrhea (Walker, 1994).

Currently obesity is not considered a mental disorder and consequently is not included among eating disorders. According to this, we will not review in this article the role of the endocannabinoid system in obesity. However, we will briefly analyse the emerging findings linking obesity and changes in the brain of obese people.

There are increasing studies analyzing specific changes, both at the molecular and the population level, in the brain of obese people. For example, reduced striatal D2 receptor availability has been found in obese patients (Volkow et al., 2008). Likewise, and maybe reflecting direct central consequences of obesity, it is noteworthy the high incidence of anxiety and depression (also present in classical eating disorders) in obese people, with a ~25% association rate between mood disorder and obesity (Simon et al., 2006). Also deserving greater consideration are the striking similarities in the pathophysiological sequel occurring with obesity and addiction what indeed suggest a re-evaluation of how these diseases are classified (Volkow and Wise, 2005). The detection in obese patients of specific changes in brain areas involved in the rewarding aspects of food has led to the model of "food addiction". However, this concept is still a matter of debate and, specially, the concern about the prevalence of food addiction and their impact on obesity (Davis et al., 2011, Meule, 2011). Several questionnaires have been developed to assess food addiction. They include the "3 C's" of addiction (compulsive use, attempts to cut down and continued use despite consequences) among others questions (Cassin and von Ranson, 2007, Gearhardt et al., 2009, Merlo et al., 2009). The results of these studies suggest that food addiction is strongly increased in obese people with a prevalence ranging from 25 to 40% and positively associated with BMI (Davis et al., 2011, Meule, 2011). However, prevalence rates suggest that the obesity epidemic cannot be fully attributed to food addiction [reviewed in (Meule,

2011)]. However, given the high prevalence of obesity, more studies are needed in order to fully understand the role of food addiction in this disease.

According to the actual classification of eating disorders, BED, AN and BN are considered chronic and disabling conditions characterized by aberrant patterns of feeding behaviour and weight regulation, including abnormal attitudes and perceptions toward body weight and shape (Kaye, 2008). Indeed, AN has the highest mortality rate among psychiatric diseases (Lowe et al., 2001). The aetiologies of these diseases are at present poorly understood, but both AN and BN occur most frequently in adolescent females. This increased incidence and prevalence may very well be a direct reflection of cultural pressures for thinness (Strober et al., 1995). However, the discrete occurrence and heritability suggest there are some biological vulnerabilities involved in these diseases (Kaye, 2008). In fact, twin studies on AN and BN suggest there is a 50-80% genetic contribution to these diseases (Bulik et al., 1998, Klump et al., 2001). However, there is little knowledge about the connection between psychological symptoms and the neuropathophysiology associated with these diseases and on how such genetic vulnerabilities impact on brain pathways and what systems are primarily involved. Because of the psychiatric nature of these diseases, the monoamine systems (i.e., the serotonin, dopamine and norepinephrine pathways) have been explored in greater detail. Among these, the serotoninergic system maybe the more adversely affected and its deregulation is present in AN patients. However, the response to selective serotonin reuptake inhibitors is variable among patients suffering different subtypes of the illness, and the efficacy of such medication has been also questioned due to the common occurrence of relapse (Kaye et al., 2001, Walsh et al., 2006).

Current research on eating disorders also points to a deregulation of neuronal circuits involved in food intake, including those related to emotional and reward

pathways linked to feeding behaviour (Stoving et al., 2009). In particular, a deranged leptin signalling system has been found in AN and BN (Monteleone et al., 2004, Holtkamp et al., 2006) and it has been hypothesized that the reward systems could be compromised leading to food intake-related dysphoria that would promote a vicious cycle of decreasing eating in order to avoid the dysphoric consequences of food consumption (Kaye, 2008). In this context, the reward system could have an important role since it integrates "liking" (pleasure/palatability) and "wanting" (appetite/incentive motivation) perceptions associated with food and, thus, AN and BN could be considered as dependency syndromes.

Evidences linking the endocannabinoid system and eating disorders

*Preclinical studies in animal model*s

The development of adequate animal models mimicking the behavioral changes that takes place in human eating disorders is currently a huge challenge in the research field of these pathologies. There is only one validated model that specifically mirrors AN. Activity-based anorexia (ABA) is a rodent paradigm which combines food restriction with free access to a running wheel. Paradoxically, whilst animals on a restricted feeding schedule or access to running wheels alone can avoid body weight loss, the combination of both factors leads to the development of a profound reduction in body weight with the expression of other anorexia-like symptoms, primarily in female rodents (Pirke et al., 1993). This animal model has been used to check the ability of some compounds to reverse ABA, especially in rats, though only modest improvements have been reached, with none of them being able to reverse the pathological condition. In spite of the important role of the eCB system in promoting food intake and the increasing evidences supporting functional endocannabinoid alterations and

polymorphisms in endocannabinoid genes in eating disorder's patients (see next sections), there is only a couple of recent studies investigating the eCB system in the ABA model and they are specifically focused on the potential therapeutic value of cannabinoids. One of these studies showed the ability of both THC and the endocannabinoid uptake inhibitor OMDM-2 in increasing food intake in a mice ABA model, though they were unable to reverse the weight loss (Lewis and Brett, 2010). Limitations of this study comprised the transient nature of the wheel running activity in mice versus the rat ABA model, possibly reflecting different species-specific strategies to survive periods of starvation, the higher mortality rate in THC-treated mice, possibly as a consequence of a hypothermic effect that could be related to a compromised thermoregulatory system in food-restricted animals and the use of only one dose of THC, thus being unexplored putative biphasic or triphasic responses that could help identifying therapeutic doses (Lewis and Brett, 2010). Interestingly, a more recent study using the rat ABA paradigm has checked different THC doses in combination with chow or high-fat diet. The authors found that 2mg/kg/day THC is capable of reducing body weight loss, shifting thermogenesis and lipid metabolism parameters towards reduced energy expenditure and lipolysis (Verty et al., 2011). Indeed, this dose of THC in combination with high-fat diet promoted a more robust response with increased food intake, decreased wheel running activity and, consequently, decreased body weight loss *via* a mechanism involving reduced energy expenditure (Verty et al., 2011). These scarce and recent preclinical data warrant more studies of the eCB system in the ABA model of anorexia nervosa.

Alterations of endocannabinoid system components in human subjects

The widespread role of the eCB system in regulating energy balance has spawned investigations into putative defects in endocannabinoid signalling that may underlie eating disorders. Increased blood levels of the endocannabinoid AEA have been found in both AN and BED patients, but not in BN patients (Monteleone et al., 2005). Indeed, AEA levels were significantly and inversely correlated with plasma leptin concentrations in both healthy controls and anorexic women. Interestingly, there is evidence to suggest that hypoleptinemia in AN patients may be an important factor underlying the excessive physical activity (Holtkamp et al., 2006), one of the hallmarks in AN. Thus, these results suggest that alterations in the eCB system associated with deregulated leptin signalling could be involved in the pathophysiology of AN. It is well-known that the eCB system and leptin interact functionally at the molecular level [reviewed in (Bermudez-Silva et al., 2012)], and thus it is easy to draw a theoretical frame in support of the important role played by both systems in AN and the therapeutic potential of leptin and cannabinoids in this disease (Stoving et al., 2009). Furthermore, elevated levels of CB1R but not CB2R mRNA have been found in the blood of females with AN and BN, further supporting the hypothesis of deregulated endocannabinoid signalling in eating disorders (Frieling et al., 2009). Paradoxically, these authors found an association between lower CB1R expression and more severe forms of the disorders. AEA belongs to the lipid family of acylethanolamides. Another member of this group of lipids, named oleoylethanolamide, has also an important role on energy balance by promoting satiety and lipolysis through the activation of the peroxisome proliferator-activated receptor-α, PPAR-α (Fu et al., 2003). This molecule has an anorexigenic action by inducing oxytocin expression in the paraventricular nucleus of the hypothalamus and, interestingly, preliminary clinical results have shown altered levels

of oleoylethanolamide in the cerebrospinal fluid and plasma of subjects recovered from eating disorders (Gaetani et al., 2008). These preliminary observations could extend the findings of altered levels of endocannabinoids in eating disorders to a more general involvement of acylethanolamides.

Human genetic association studies

Given the important contribution of genetics to AN and BN (in fact, the heritability estimates are similar to disorders typically viewed as biological like schizophrenia and bipolar disorder) human genetic association studies have been performed in order to identify genes involved in these pathologies, including genes belonging to the eCB system. Among these, CNR1 and CNR2 (the genes encoding cannabinoid CB1Rs and CB2Rs, respectively), as well as the genes encoding the main enzyme responsible in the degradation of AEA (FAAH), NAAA (N-Acylethanolamine-hydrolyzing acid amidase, which functions similar to FAAH but has a different optimal pH) and MAGL have been studied. The first family-based study involved fifty two families (parents with one or two affected siblings) that were genotyped for the (AAT) trinucleotide repeat of CNR1 gene. The distribution of alleles transmitted to the patients was not found to be significantly different from the non-transmitted parental alleles. However, upon dividing the samples to restricting and binging/purging subtypes of AN, the data analysis revealed a preferential transmission of different alleles in each of the subtypes, suggesting restricting AN and binging/purging AN may be associated with different alleles of the CNR1 gene (Siegfried et al., 2004). However, a subsequent study involving up to 91 German AN trios (patient with AN and both biological parents) was unable to confirm these results, nor did it show an association for any of 15 single nucleotide polymorphisms representative of regions with restricted haplotype diversity

in FAAH, NAAA and MAGL genes (Muller et al., 2008). Another study in 115 overweight/obese subjects with binge eating disorder, 74 non- binge eating disorder patients with obesity and 110 normal weight healthy controls investigated one of these FAAH polymorphisms, previously implicated in obesity in binge-eating disorder, and reporting a lack of association (Monteleone et al., 2008) and in a more recent article these authors studied the association of this FAAH polymorphism and the CNR1 polymorphism in both AN and BN, in 134 patients with AN, 180 patients with BN and 148 normal weight healthy controls (Monteleone et al., 2009). The authors found a significant increase in the frequency of both polymorphisms in AN and BN patients, a result in sharp contrast with the previous findings by Muller and collaborators (2008) that showed a lack of association of these polymorphisms with AN. Additionally, Monteleone and collaborators (2009) found a synergistic effect of the two polymorphisms in AN but not in BN. Finally, a recent article has detected an association of a CNR2 polymorphism with both AN and BN (Ishiguro et al., 2010) in a study comprising in 204 subjects with eating disorders and 1876 healthy volunteers in Japanese population. Taken together, the human genetic association studies show evidence of association between eCB system genes and eating disorders, but further studies are necessary to definitively confirm these findings.

Toward a cannabinoid-based therapy in eating disorders

Cannabis preparations have been used for both medicinal and recreational purposes for centuries. Its ancient medicinal use has been primarily related to ameliorate pain and increase appetite in disease states. However, because of their psychostimulant properties and the lack of an adequate body of knowledge, their use in western medicine has been excluded until recently. During the last 20 years this picture has dramatically changed.

There has been an exponential increase in the knowledge of the molecular mechanisms underlying cannabinoid effects, and morphological, physiological and pathophysiological studies have shown that the molecular system supporting these effects (i.e., the eCB system), is ubiquitous and has a highly relevant role in maintaining whole body homeostasis and, especially, energy homeostasis (Matias and Di Marzo, 2007). This fact has led to an increased interest in the medical use of cannabinoid-related drugs. Thus, in 1985 the Food and Drug Administration approved Marinol® (dronabinol), a synthetically-derived THC (the main psychoactive constituent of cannabis) preparation, to relieve nausea and vomiting associated with chemotherapy in cancer patients who have failed to respond adequately to other antiemetics, and in 1992 this compound was also approved for inducing appetite in AIDS patients suffering from cachexia (Nelson et al., 1994, Beal et al., 1995). Similarly, Nabilone® (a synthetic cannabinoid that mimics THC) was also approved in 1985 for ameliorating the nausea of cancer chemotherapy. A more controversial step forward was the use of a cannabinoid CB1R antagonist/inverse agonist (rimonabant) for management of complicated obesity. Although the Food and Drug Administration never approved this drug, the European Medicine Agency did and Acomplia® (the commercial name of rimonabant) was in the market for approximately 2 years. Despite the weight loss and improved cardiometabolic profile observed in obese patients, the drug had to be removed from the market due to its undesirable central side effects [reviewed in (Bermudez-Silva et al., 2010)]. More recently, Sativex® (the combination of THC and CBD) has been marketed in Canada, New Zeeland and European countries like the United Kingdom, Germany, Denmark and Spain for the treatment of spasticity due to multiple sclerosis, and it is currently in phase III clinical development for the treatment

of cancer pain. It has been also approved and marketed in Canada for the relief of neuropathic pain in multiple sclerosis and cancer pain.

Taken into account the good therapeutic management of cannabinoids in cachexia and malnutrition associated with cancer and AIDS, it looks feasible that this kind of pharmacotherapy could be also useful in the treatment of eating disorders. Unfortunately, there are only two small trials assessing cannabinoid treatment in AN [reviewed in (Stoving et al., 2009)]. The former involved 11 AN patients in a four-week crossover trial and THC treatment resulted in increased sleep disturbances and interpersonal sensitivity, whereas there was no significant effect on weight gain (Gross et al., 1983). Unfortunately, this study raised several concerns given it was an in-patient study and the occasional tube feeding was used. In addition, THC was compared to diazepam instead of placebo, which could be a confounding factor given diazepam has also been reported to increase food intake *per se* (Naruse et al., 1991). The latter involved nine AN out-patients treated with THC. The results showed a significant improvement of depression and perfectionism scores without improving weight gain (Berry, 2006).

Currently, there is an ongoing phase III clinical trial involving 22 subjects to reveal if severe chronic AN patients treated with Marinol® have significant improvement on weight, with secondary objectives of the study being evaluation of eating disorder inventory scale, motor and inner restlessness and endocrine parameters (https://www.clinicaltrialsregister.eu; EudraCT Number: 2007-005631-29). With this very limited number of performed trials (the last one being still not finished) it seems clear that no conclusions can be drawn out regarding the therapeutic validity of a cannabinoid-based approach in eating disorders. However, the satisfactory clinical use

of cannabinoid agonists in other pathologies demands and encourages the development of further clinical trials on eating disorders patients.

A new avenue of research in this field is the finding that non-THC phytocannabinoids are also able to stimulate feeding, thus suggesting that some phytocannabinoids or cannabis extracts lacking the main psychotropic constituent, and consequently avoiding the undesired psychotropic effects of full cannabis extracts, could be useful in medical conditions where an stimulation of appetite is desired (Farrimond et al., 2012).

Conclusions

The eCB system is a lipid signalling system comprising all the molecular machinery needed to properly activate the cannabinoid receptors. Regarding energy homeostasis, the eCB system is strategically located in all the key points involved in food intake and energy expenditure. It is perhaps one of the few that can coordinate all the players involved in energy balance, thus being an interesting target in all the diseases related to an imbalanced energy homeostasis like obesity and eating disorders. The aetiology of eating disorders is currently unknown and the molecular systems involved are still largely a mystery. However, an increasing body of evidence points to an important role of brain circuits related with feeding behaviour, especially those related with the reward system, where the eCB system has an important role. Recent findings, starting from human genetic association studies and including other molecular, physiological and pathophysiological studies, are suggestive of a deregulation of the eCB system in eating disorders that is still not completely understood. In clinical practice cannabinoid agonists are being used safely and successfully in other diseases in which weight gain is needed, like cachexia in AIDS patients but, however, the trials on the therapeutic

validity of cannabinoids on eating disorders are very scarce and the results are inconclusive. Taken together, all these considerations encourage the development of new clinical trials for assessing cannabinoid agonists in the management of eating disorders.

Acknowledgements: This work was supported by Instituto de Salud Carlos III (PI 10/02308), Redes temáticas de Investigación Cooperativa en salud (ISCIII y FEDER): RD06/0001/1013, Ministerio de Ciencia e Innovación: BFU2009-10109, GRUPOS UCM-BSCH (951579). Plan Nacional sobre Drogas SAS/1250/2009. FJBS has a research contract with the National System of Health, Ministerio de Sanidad, Gobierno de España (Instituto de Salud Carlos III; CP07/00283).

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