The role of the endocannabinoid system in eating disorders: pharmacological implications

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The endocannabinoid (eCB) system is a widespread intercellular signalling mechanism that plays a critical role in body homoeostasis. 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 come to be seen as an interesting target for the management of diseases characterized by an imbalanced energy homoeostasis, such as 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, such as 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, for example 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. *Behavioural Pharmacology* 23:526–536 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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A brief update on the endocannabinoid system

Derivatives of *Camabis sativa*, such as marijuana and hashish, as well as Δ -9-tetrahydrocannabinol (THC), the major psychoactive component of the plant, have long been known to stimulate appetite (Foltin *et al.*, 1986). The identification of specific binding sites for THC in the central nervous system allowed the identification of an endogenous endocannabinoid (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, type 1 (CB1R) (Herkenham *et al.*, 1991), and type 2 (CB2R) (Munro *et al.*, 1993). Endocannabinoids, because of their lipophilic nature, are synthesized and released 'on demand' by the cleavage of membrane phospholipid precursors. The 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 is synthesized from membrane *N*-arachidonoyl-phosphatidylethanolamine (*N*-arachPE) through the activity of

a phospholipase D (NAPE-PLD) (Okamoto et al., 2009) or by a two-enzyme pathway that involves an $\alpha\beta$ -hydrolase (ABH4) and a glycerophosphodiesterase (GDE1; Simon and Cravatt, 2008), whereas 2-AG is synthesized through a two-enzyme cascade of phospholipase C (PLC) and diacylglycerol lipase (DGL; Bisogno et al., 2003). The activity of endocannabinoids is terminated by their inactivation. As a first step, endocannabinoids can passively diffuse through lipid membranes, although a high-affinity transporter - not yet identified - seems to accelerate this process. AEA degradation is mediated by different enzymes: a fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), a lysosome-localized fatty acyl amide hydrolase with an acid optimum (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 induced by the enzyme monoacylglycerol lipase (MGL), with limited contributions from an $\alpha\beta$ -hydrolase domain containing (ABHD) 6 and 12 hydrolases (Blankman et al., 2007). Notwithstanding, both 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 agonists of the CB1R and CB2R cannabinoid receptors. CB1Rs are expressed ubiquitously

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throughout the brain (see Mackie, 2005 for a review). CB2Rs, initially proposed to be mainly in the periphery and associated with the immune system (Munro et al., 1993; Galiegue et al., 1995), have been described in a diversity of locations within the body (Roche et al., 2006; Liu et al., 2009), and more recently in the brain (Van Sickle et al., 2005; Gong et al., 2006; Onaivi, 2006). 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 a review). For example, AEA is also able to activate the transient receptor potential vanilloid type 1 (TRPV1) ion channel (Starowicz et al., 2007), two G-protein-coupled receptors, GPR55 and GPR119, have been proposed as novel potential cannabinoid receptors (Baker et al., 2006), and increasing evidence now suggests that endocannabinoids are also natural activators of the peroxisome proliferatoractivated receptor family of nuclear receptors (O'Sullivan and Kendall, 2010).

Despite the fact that our knowledge of the molecular architecture of the eCB system has expanded rapidly in recent decades, the physiology, concentration and regionalized distribution of its components remain largely elusive. In recent years, the eCB system has come to be seen as a widespread modulatory system involved in the homoeostatic control of a plethora of physiological functions, including food intake and energy expenditure. This eCB system is believed to be usually silent and to become transiently activated after exposure to stressful conditions. Accordingly, increasing evidence supports the view that the activation of the eCB system plays important regulatory roles in the control of food consumption, energy balance, fat storage and body mass through central and peripheral mechanisms at multiple levels including the brain, gastrointestinal tract, liver, pancreas, muscle and adipose tissue (for recent reviews, see De Kloet and Woods, 2009; Andre and Gonthier, 2010; Maccarrone et al., 2010).

The endocannabinoid system in energy homoeostasis

The hypothalamus is a key brain structure involved in energy balance regulation. The arcuate nucleus (ARC) of the hypothalamus contains a specific set of neurons that sense hormonal and nutrient signals coming from the periphery (Cota et al., 2007). Orexigenic signals activate a subpopulation of ARC neurons coexpressing neuropeptide Y (NPY) and agouti-related peptide (AgRP), whereas anorexigenic signals activate a subpopulation of ARC neurons coexpressing pro-opiomelanocortin (POMC) and cocaine-regulated and amphetamine-regulated transcript (CART). Simultaneously, anorexigenic signals inhibit NPY/ AgRP neurons (Cone, 2005). Thus, some peripheral signals such as leptin are potent suppressors of feeding by stimulating anorexigenic POMC/CART neurons while

reciprocally inhibiting or xigenic NPY/AgRP neurons. The ARC neuropeptides POMC and AgRP oppositely regulate melanocortin receptors located in the paraventricular nucleus (PVN). Melanocortins activate these receptors, leading to decreased food intake, whereas AgRP acts as an endogenous antagonist of melanocortin receptors, thus promoting food intake. Glucocorticoids, which show orexigenic properties, can reach the PVN and, through specific membrane receptors, modulate neuroendocrine responses involved in stress and energy balance (Tasker, 2006). Indeed, orexins and melanin-concentrating hormone neurons located in the LH play a role in the regulation of energy balance (Morrison and Berthoud, 2007) and they are also interconnected with the PVN and ARC. Furthermore, the hypothalamus is connected with both the brainstem (which detects and responds to hunger and satiety signals) and the brain reward system (which modulates the motivation to obtain food). Despite the low expression of CB1R in the hypothalamus, a number of studies have shown that endocannabinoids, through CB1Rs, exert a profound influence on the hypothalamic regulation of food intake (reviewed by Bermudez-Silva et al., 2012). In fact, the intrahypothalamic administration of endocannabinoids or CB1R agonists increases food intake in rodents, an effect that is prevented by pretreatment of the animals with the CB1R antagonist rimonabant (Cota, 2007). CB1Rs have been reported in GABAergic terminals entering the ARC, and POMC cells produce endocannabinoids, which inhibit GABA release onto POMC neurons (Hentges et al., 2005). Similarly, in the PVN, the glucocorticoid-induced suppression of glutamatergic inputs is mediated through the retrograde release of endocannabinoids and CB1R activation (Tasker, 2006). Similarly, in the LH, the ability of orexins and MCH to modulate neuronal excitability seems to be mediated by retrograde endocannabinoid signalling (Haj-Dahmane and Shen, 2005). The role of the CB1R in the hypothalamic leptin-mediated anorectic effects is also important (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 that regulate and control behaviour by inducing pleasurable effects. The major rewarding pathway in the brain is the mesolimbic pathway that projects from the ventral tegmental area (VTA) through the medial forebrain bundle to the nucleus accumbens, which is the primary release site for the brain's main reward signal, the neurotransmitter dopamine. CB1Rs are expressed in presynaptic glutamatergic and GABAergic nerve terminals in the VTA, and endocannabinoids are synthesized by VTA dopamine neurons, playing a role in the finetuned regulation of these cells (Maldonado et al., 2006). Although it is still unclear exactly which cell populations express CB1Rs in the nucleus accumbens, it seems that

endocannabinoids within this area can increase food intake in a CB1R-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 to the eCB system (for reviews, see Di Marzo et al., 2009; Bermudez-Silva et al., 2010). 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 by Di Marzo et al., 2009).

In terms of the peripheral regulation of food intake by endocannabinoids, it has been found that endocannabinoids coming from the gastrointestinal tract are able to modulate feeding by acting on peripheral sensory terminals (Gómez et al., 2002). Food deprivation increased the anandamide content in the small intestine, but not in the brain or the stomach, and refeeding normalized intestinal anandamide levels. Indeed, peripheral but not central administration of anandamide induced hyperphagia in partially satiated rats, with capsaicin deafferentation abolishing anandamide effects (Gómez et al., 2002). Interestingly, the gut endocannabinoid system has been involved in the positive feedback mechanism that drives fat intake. Using a sham-feeding paradigm, which isolates orosensory from the postingestive influences of foods, DiPatrizio et al. (2011) showed that the orosensory properties of fat, through the vagus nerve, mobilize 2-AG and AEA in the intestine, and localized functional blockade of this system suppressed fat sham feeding. These findings have important implications in the development of future strategies aimed at selectively reducing the overeating of fatty foods. Also in agreement with an important role of AEA in food intake, a recent human study showed that anandamide has a circulating profile consistent with a role as a physiological meal initiator (Gatta-Cherifi et al., 2011). Just before the meal, the plasma levels of AEA increased significantly, and 1 h after the meal, AEA levels decreased. Interestingly, the authors did not find changes in 2-AG plasma levels, suggesting that AEA might be of physiological relevance in the regulation of human eating behaviour (Gatta-Cherifi et al., 2011).

In conclusion, the eCB system is located strategically in all the key points involved in food intake and energy expenditure, both at the central and at the peripheral level. Thus, it is perhaps one of the few systems that can coordinate all the players involved in energy balance (for reviews, see Pagotto et al., 2006; Matias and Di Marzo, 2007). Together with its action on peripheral tissues, the eCB system influences feeding behaviour at the central nervous system level by acting on circuits located in the hypothalamus, the reward system and the brainstem, with the overall net effect being anabolic (for reviews, see De Kloet and Woods, 2009; Di Marzo et al., 2009; Andre and Gonthier, 2010; Maccarrone et al., 2010).

Pathophysiology of eating disorders

Food intake or eating is the process by which edible substances are consumed by living creatures to balance the energy expenditure. This process relies on physiological mechanisms regulating appetite and the natural drive to eat. Under 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, that is 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 because of embarrassment; and feeling of disgust, depression or guilt after overeating. Criteria include occurrence on average on 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 (American Psychiatric Association, 1994). BN is characterized by a cycle of binge eating, followed by purging to prevent weight gain. Purging methods often include selfinduced vomiting, use of laxatives or diuretics, excessive exercise and fasting. AN is characterized by 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 amenorrhoea (Walker, 1994).

Currently, obesity is not considered a mental disorder and consequently is not included among eating disorders. Accordingly, in this article, we will not review the role of the endocannabinoid system in obesity. However, it should be noted that there are increasing studies analysing specific changes, both at the molecular and at the population level, in the brains of obese individuals. For example, reduced striatal D2 receptor availability has been found in obese patients (Volkow et al., 2008). Similarly, and perhaps reflecting the direct central consequences of obesity, it is noteworthy that there is a high incidence of anxiety and depression (also present in classical eating disorders) in obese individuals, affecting around 50% of this population. Also deserving greater consideration are the striking similarities in the pathophysiological sequel occurring with obesity and addiction. Therefore, it appears that there are sufficient arguments to suggest a re-evaluation of obesity as an eating disorder (Volkow and Wise, 2005).

BED, AN and BN are considered chronic and disabling conditions characterized by aberrant patterns of feeding behaviour and weight regulation, including abnormal attitudes to and perceptions of body weight and shape (Kave, 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. There are studies relating eating disorders to environmental stressful conditions. Thus, cultural pressures for thinness may very well be related to the increased incidence and prevalence of these diseases (Strober et al., 1995). Indeed, one study has suggested that women with post-traumatic stress disorder, especially because of sexually related trauma, are more likely to develop AN (Reyes-Rodríguez et al., 2011). However, the discrete occurrence and heritability suggest that there are also some biological vulnerabilities involved in these diseases (Kaye, 2008). In fact, twin studies on AN and BN suggest that there is a 50-80% genetic contribution to these diseases (Bulik et al., 1998; Klump et al., 2001). Girls with attention deficit hyperactivity disorder (a disease that is believed to have around 75% genetic contribution) have been reported to have a greater chance of contracting an eating disorder than those not affected by this disease (Biederman et al., 2007).

Unfortunately, there is little knowledge about the link between psychological symptoms and the neuropathophysiology associated with these diseases, and on how such genetic vulnerabilities impact on brain pathways and which 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 than others. Theoretically, serotonin disturbances could contribute towards the deregulation of appetite and compulsive and anxiety-related behaviours (Blundell, 1984; Lucki, 1998). Accordingly, deregulation of the serotoninergic system is present in AN and BN patients (Jimerson et al., 1997; Kaye et al., 2004). Moreover, a recent functional neuroimaging study has shown cortical metabolic dysfunction and changes in monoamine neurotransmitter systems in AN patients (Van Kuyck et al., 2009). However, the response to selective serotonin reuptake inhibitors is variable among patients with different subtypes of the illness, and the efficacy of such medication has also been questioned because of the common occurrence of relapse (Kaye et al., 2001; Walsh et al., 2006).

Current research on eating disorders also points towards a deregulation of neuronal circuits involved in homoeostatic and hedonic aspects of food intake (Stoving et al., 2009). Leptin and ghrelin are two peripheral hormones involved in the homoeostatic control of food intake and they transmit important information on the energy status of the body to the brain. Leptin is synthesized by the adipocytes and

reaches the hypothalamus to activate specific neuronal populations that suppress food intake and increase the metabolic rate. In contrast, ghrelin is a stomach-derived peptide that stimulates food intake and energy storage (Zigman and Elmquist, 2003). Its orexigenic effect is mediated through the activation of NPY/AgRP neurons in the arcuate hypothalamic nucleus. A deranged leptin signalling system has been found in AN and BN (Jimerson et al., 2000; Monteleone et al., 2000; Holtkamp et al., 2006), suggesting that neuronal circuits modulated by leptin could participate in the pathophysiology of these diseases. Indeed, ghrelin has been linked to the pathophysiology of Prader-Willi syndrome, an uncommon genetic disorder associated with elevated food intake (Cummings et al., 2002; DelParigi et al., 2002), but its role in BN or BED is not clear (Troisi et al., 2005).

Interestingly, ghrelin has also been involved in chronicstress-related changes in feeding and body weight homoeostasis (Lutter et al., 2008), and leptin has been found to be decreased in mice subjected to chronic mild stress (Lu et al., 2006). These findings indicate molecular targets and signalling systems that could be involved in the aetiology/ pathophysiology of stress-related eating disorders.

Emerging data support the notion that leptin and ghrelin can also modulate the brain reward system and, consequently, the hedonic aspects of food intake (Lutter and Nestler, 2009). In this context, the reward system could play an important role as it integrates 'liking' (pleasure/ palatability) and 'wanting' (appetite/incentive motivation) perceptions associated with food. Interestingly, BN and BED include compulsive food intake as a part of the clinical syndrome, suggesting alterations in the mesolimbic dopamine signalling system. Unfortunately, very little is known about the pathophysiology of compulsive food intake in BN and BED patients. Only two neuroimaging studies have been published, showing abnormal activation of the anterior cingulate cortex in BN patients (Frank et al., 2006; Peñas-Lledó et al., 2007). The mechanisms responsible for the abnormal limbic activation in these patients are unknown, but they could include deranged peripheral leptin and ghrelin levels.

Food addiction has been defined as 'a loss of control over food intake' and a growing body of evidence supports this concept by showing that limbic regions experience similar neuroadaptations after exposure to both food and drug rewards. Indeed, these adaptations modify the motivation to obtain both types of reward (Lutter and Nestler, 2009). From this perspective, AN, BN and BED could be considered as dependency syndromes. It has been hypothesized that the reward systems could be compromised in AN, leading to food intake-related dysphoria that would promote a vicious cycle of decreasing eating to avoid the dysphoric consequences of food consumption (Kaye, 2008). In contrast, BN and BED, in a manner similar to drugs of abuse, could be related to food intake-euphoria that would promote a vicious cycle of increasing eating to avoid the dysphoric consequences of fasting.

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 (Cassin and von Ranson, 2007; Gearhardt et al., 2009; Merlo et al., 2009). Some studies have investigated the relationship between food addiction and BED (reviewed in Meule, 2011). The results of these studies suggest that food addiction is strongly increased in individuals with BED, with as many as 92.4% being classified as food addicted when modified DSM-IV substance dependence criteria were used (Cassin and von Ranson, 2007). However, 40.5% of individuals with BED were diagnosed as having food addiction after applying modified Goodman's addictive disorder criteria (Cassin and von Ranson, 2007). Using a different scale (the Yale Food Addiction Scale), 56.8% of obese individuals with BED were classified as having food addiction (Gearhardt et al., 2012).

Evidence linking the endocannabinoid system and eating disorders

Preclinical studies in animal models

The development of adequate animal models mimicking the behavioural changes that take place in human eating disorders is currently a huge challenge in the research field of these pathologies. Because of the complex nature of eating disorders, current animal models can only provide some characteristic traits of the human disease. Several procedures have been used as animal models of AN (reviewed in Kim, 2012). There are some genetic models of AN, the most commonly used being the anx/ anx mouse. These mice have decreased food intake behaviour, reduced body weight, reduced serum leptin levels and hypothalamic degeneration. However, this latter finding is a concern, as individuals with AN feel hunger and yet refuse to eat, unlike anx/anx mice. Other genetic models of AN include mice lacking genes such as BDNF, tyrosine hydroxylase, δ-opioid receptor, specific serotonin receptors or M3 muscarinic receptors, all of them being genes involved in feeding behaviour (reviewed in Kim, 2012). Stress models have also been used because stress can induce weight loss and contribute toward loss of appetite. However, a disadvantage of this model is that stress induction can harm the animals physically. Dietary restriction can also be used as an AN model, given that many of the changes reported in AN can be mimicked in mice by dietary restriction alone. A drawback of this model is that food restriction is not voluntary, unlike the situation in patients with this disease. Self-motivated caloric restriction is characteristic of AN and, perhaps because of this, the self-starvation/ activity-based anorexia (ABA) model is considered the most robust animal model of AN. ABA is a rodent paradigm that combines food restriction with free access

to a running wheel. Paradoxically, although 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 considerable 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, although only modest improvements have been achieved, with none of them being able to reverse the pathological condition.

Despite the important role of the eCB system in promoting food intake and the increasing evidence supporting functional endocannabinoid alterations and polymorphisms in endocannabinoid genes in patients with eating disorders (see next sections), only a few recent studies have investigated 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 to increase food intake in a mouse ABA model, although they were unable to reverse the weight loss (Lewis and Brett, 2010). The limitations of this study included the following: (a) the transient nature of the wheel-running activity in mice, in contrast to the rat ABA model, possibly reflecting different species-specific strategies to survive periods of starvation, (b) 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 (c) the use of only one dose of THC, thus leaving unexplored putative biphasic or triphasic responses that could help in the identification of therapeutic doses (Lewis and Brett, 2010). Interestingly, a more recent study using the rat ABA paradigm has examined different THC doses in combination with chow or a high-fat diet. The authors found that 2 mg/kg/day THC reduced body weight loss, and shifted thermogenesis and lipid metabolism parameters towards reduced energy expenditure and lipolysis (Verty et al., 2011). Indeed, this dose of THC in combination with the high-fat diet promoted a more robust response, with increased food intake, decreased wheelrunning activity and, consequently, decreased body weight loss through 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 AN.

Alterations in endocannabinoid system components in humans

The widespread role of the eCB system in regulating energy balance has led to investigations into putative defects in endocannabinoid signalling that may underlie eating disorders. Increased blood levels of the endocannabinoid AEA but not 2-AG have been found in both AN and BED patients, but not in BN patients (Monteleone et al., 2005).

However, the authors found no changes in the blood mRNA levels of either CB1R or CB2R. In terms of leptin levels, AN patients showed significantly decreased circulating levels and women with BED had significantly enhanced plasma levels. No changes were found in BN patients. These leptin data are in agreement with the literature and it has even been proposed that hypoleptinaemia in AN patients may be an important factor underlying the excessive physical activity (Holtkamp et al., 2006), one of the hallmarks of AN. Furthermore, AEA levels were significantly and inversely correlated with plasma leptin concentrations in both healthy controls and anorexic women, but not in BN and BED patients (Monteleone et al., 2005). However, no significant correlations were found between endocannabinoids and body weight, BMI, body fat mass and body lean mass. Similarly, no correlations were found between endocannabinoids and age, duration of the illness and illness scores (Monteleone et al., 2005). The authors interpreted their findings on the basis of the known relationship between AEA and leptin (Di Marzo et al., 2001), suggesting that increased levels of AEA are secondary to a leptin deficiency. The apparent contradiction in the BED patients can be attributed to the obese phenotype of these patients, who may have possibly had hyperleptinaemia because of impaired leptin signalling (Monteleone et al., 2005). In addition, increased levels of AEA could represent an adaptive response aiming at counteracting the restrictive behaviour in AN patients, which, however, would be unsuccessful, with the psychological factors overwhelming the biological hunger signal. Nevertheless, the increased AEA in patients with BED would enhance the drive to eat, eventually leading to their binge-eating behaviour (Monteleone et al., 2005). Finally, given the role of endocannabinoids in controlling the brain reward system, the authors also suggested that increased AEA levels could be mediating the rewarding aspects of the aberrant eating behaviour of both AN and BED patients (Monteleone et al., 2005). In general, these findings challenge the hypothesis of a hypoactive eCB system in anorectic conditions (Van der Stelt and Di Marzo, 2003; Di Marzo, 2008a, 2008b), and suggest that cannabinoid agonists could increase appetite in these patients. In fact, although scarce and with significant limitations, the clinical trials that have been performed showed no promising results (see the next section for a full discussion of this topic). An important limitation of this study is that only plasma levels of endocannabinoids were measured and the eCB system has been described as a local signalling system, which raises a question as to what extent changes in blood levels of endocannabinoids reflect changes in the brain.

The levels of CB1R and CB2R expression have been also analysed in patients with eating disorders. Whereas there were no changes in the first study (Monteleone et al., 2005), perhaps because of a small sample size (n = 5 per group), a second study detected increases in CB1R but not CB2R mRNA levels in the blood of both AN and BN patients when compared with the controls, with no significant difference between AN and BN patients (Frieling et al., 2009). This study included 20 women with AN, 23 women with BN and 26 healthy women. The authors also divided the study sample according to the presence of binging behaviour and purging behaviour, but they found no differences in CB1R or CB2R mRNA levels when comparing binging with no binging or purging with no purging. The increased CB1R mRNA levels in patients with eating disorders, which are counterintuitive to the notion that elevated levels of endocannabinoids downregulate CB1R expression, were considered to be a compensation to a hypothetical reduced receptor sensitivity or, alternatively, a physiological consequence of elevated endocannabinoid levels in these disorders (Frieling et al., 2009). Paradoxically, an association was found between lower CB1R expression and more severe forms of the disorders (Frieling et al., 2009). Given that no difference was found in CB1R mRNA between AN and BN, this association analysis included both groups of patients, and it was argued that the result was in agreement with reports describing CB1R downregulation in an animal model of attention deficit hyperactivity disorder that is associated with increased impulsivity (Frieling et al., 2009).

The development of a CB1R-specific radioligand for positron emission tomography has allowed in-vivo monitoring of CB1R availability. In a recent report, increased availability of CB1R in brain areas of AN and BN patients has been described (Gérard et al., 2011). The study showed a whole-brain increase in CB1R availability in the AN group compared with the BN patients and healthy controls. Indeed, further changes were detected on performing a regional analysis. CB1R availability was increased in the bilateral insular cortex (a key area in the neural control of interoception, that is, the sense of the body's physiological condition, reward and emotion processing) of AN and BN patients in comparison with healthy controls. Furthermore, only in AN, clustered CB1R increases were detected in the inferior frontal cortex and in the inferior temporal cortex compared with the control group (Gérard et al., 2011). Interestingly, in AN and to a much lesser extent in BN, dysfunction in frontal and temporal areas related to executive functioning and emotional processing has been observed for other in-vivo neurochemical markers (Van Kuyck et al., 2009). Taken together, all these findings suggest that alterations in the eCB system, associated with deregulated leptin signalling, could be involved in the pathophysiology of eating disorders and that, consequently, there is therapeutic potential for both leptin and cannabinoids in these diseases (Stoving et al., 2009).

Furthermore, endocannabinoids belong to a larger family of lipids called acylethanolamides. Interestingly, although endocannabinoids are orexigenic signals, other members of the endogenous acylethanolamide family include lipids

with contrasting roles, that is with anorexigenic properties, such as oleoylethanolamide. This is an anandamide analogue that does not target the cannabinoid receptors, but plays an important role in energy balance by promoting satiety and lipolysis through the activation of the peroxisome proliferator-activated receptor-α (Fu et al., 2003). This molecule has an anorexigenic action by inducing oxytocin expression in the PVN of the hypothalamus and, interestingly, preliminary clinical results have shown altered levels of oleoylethanolamide in the cerebrospinal fluid and plasma of patients who have 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 those found in disorders typically viewed as biological such as schizophrenia and bipolar disorder), human genetic association studies have been performed to identify the genes involved in these pathologies, including genes belonging to the eCB system. Among these, CNR1 and CNR2 (the genes encoding the CB1R and CB2R, respectively), as well as the genes encoding the main enzymes responsible for the degradation of endocannabinoids, that is FAAH and NAAA, which inactivate AEA, and MAGL, which degrades 2-AG, have been studied. The first family-based study involved 52 families (parents with one or two affected siblings) that were genotyped for eight alleles comprising polymorphic triplet repeats (AAT) of the CNR1 gene. Using the haplotype relative risk method, the distribution of alleles transmitted to the patients was not found to be significantly different from the nontransmitted parental alleles. However, on dividing the samples into restricting and binging/purging subtypes of AN, the data analysis indicated a preferential transmission of 13-repeat and 14-repeat alleles in each of the subtypes. The 13-repeat allele was preferentially transmitted in binge-purge type AN patients (P = 0.05) and the 14-repeat allele showed a trend towards preferential transmission in restricting-type AN patients (P = 0.07). The authors concluded that different alleles of the CNR1 gene may be associated with different types of AN (Siegfried et al., 2004).

However, a subsequent study involving up to 91 German AN trios (patients with AN and both biological parents) was unable to confirm these results (although separate analysis on the AN subgroups was not performed), nor did it show an association for any of 15 single-nucleotide polymorphisms (SNPs) representative of regions with restricted haplotype diversity in FAAH, NAAA and MAGL genes (Muller et al., 2008). Among these 15 SNPs, the authors studied several SNPs that have been related previously to obesity and/or drug abuse. For example, rs324420 in the FAAH gene decreases enzyme activity and, as such, increases the endocannabinoid level and presumably food intake (Sipe et al., 2005). Similarly, they studied the CNR1 haplotype comprising the minor alleles of SNP rs806379, rs1535255 and rs2023239 (Zhang et al., 2004) and the G allele of rs1049353 (Gazzerro et al., 2007). The transmission disequilibrium test failed to detect an association for any of the SNPs analysed and the authors concluded that these variations do not seem to play a major role in the genetic aetiology of AN in these patients (Muller et al., 2008).

Another study, involving 115 overweight/obese patients with BED, 74 non-BED patients with obesity and 110 normal-weight healthy controls, also examined the SNP rs324420 in the FAAH gene. The authors found this polymorphism to be associated with overweight/obesity, as reported previously (Sipe et al., 2005), but not with BED (Monteleone et al., 2008). In a more recent article published by the same group, the association of this FAAH polymorphism and the CNR1 polymorphism rs1049353 was studied in 134 patients with AN, 180 patients with BN and 148 normal-weight healthy controls (Monteleone et al., 2009). They found higher frequencies of the AG genotype and the A allele in the CNR1 gene and the AC genotype and the A allele in the FAAH gene, in both AN and BN patients. These results are not in agreement with those reported previously by Muller et al. (2008), who failed to find an association of these same polymorphisms in a different AN population. Interestingly, the authors found a P value of 0.11 for the A allele of the CNR1 SNP rs1049353, although further analysis could not show an association of the A allele with the AN phenotype (P = 0.27). Monteleone et al. (2009) also found a synergistic effect of the two polymorphisms in AN but not in BN. In conclusion, these authors found an association of both CNR1 and FAAH genes with eating disorders that supports a potential role of these SNPs in the biological susceptibility to develop either AN or BN.

Finally, a recent investigation has detected an association of a nonsynonymous CNR2 polymorphism with both AN and BN (Ishiguro et al., 2010). The authors studied 204 patients with eating disorders (composed of 94 AN and 111 BN) and 1876 healthy volunteers in the Japanese population. They found the R allele to be significantly more abundant in patients with eating disorders than in controls (P = 0.04). In addition, differentiation of the patients with AN and BN did not indicate a difference in allele frequency. The authors suggested that this association could reflect a role of CB2R in the endocannabinoid-mediated signalling mechanisms associated with food intake regulation and in eating disorders.

Taken together, the human genetic association studies show evidence of an association between eCB system genes and eating disorders, but further studies are necessary to definitively confirm these findings. The

mechanisms through which these SNPs may predispose to both AN and BN are not obvious. The FAAH polymorphism rs324420 has been related to deficient enzyme activity in humans (Chiang et al., 2004), and decreased activity would lead to reduced inactivation of AEA and, eventually, increased endocannabinoid tone, which may enhance the natural drive to eat. This could be the case in obese, BED and BN patients, who showed an increased frequency of this SNP (Monteleone et al., 2008, 2009), but it is counterintuitive for AN patients who show starving behaviours. Alternatively, this deregulation could be especially relevant in the brain rewarding system, reinforcing the aberrant behaviour in patients with an eating disorder, although they belong to opposite types in the eating disorder spectrum (Monteleone et al., 2009). In terms of the CNR1 rs1049353 SNP, it is striking that this polymorphism is synonymous, that is does not alter the amino acid sequence of the protein. Nevertheless, the possibility remains that this change affects the stability or the translation of the mRNA (Monteleone et al., 2009). Hypothetically, the nonsynonymous polymorphism in the CNR2 gene would lead to a less/ nonfunctional CB2R that, under normal conditions, could counteract signalling through CB1R. A higher signalling through CB1R in brain reward circuits could lead to aberrant behaviours in patients with eating disorders in a similar manner to higher endocannabinoid levels, because of deficient FAAH activity (see above).

Towards 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 for amelioration of pain and to 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 changed markedly. 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 plays a highly relevant role in maintaining whole-body homoeostasis and, especially, energy homoeostasis (Matias and Di Marzo, 2007). This has led to an increased interest in the medical use of cannabinoidrelated drugs. In 1985, the Food and Drug Administration approved Marinol (dronabinol; Solvay Pharmaceuticals Inc., Marietta, Georgia, USA), 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 with 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 CB1R antagonist/inverse agonist (rimonabant) for the 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; Sanofi-Aventis Groupe, Paris, France) was in the market for approximately 2 years. Despite the weight loss and the improved cardiometabolic profile observed in obese patients, the drug had to be removed from the market because of its undesirable central side-effects (reviewed in Bermudez-Silva et al., 2010). More recently, Sativex (a combination of THC and cannabidiol; GW Pharmaceuticals plc, Salisbury, UK) has been marketed in Canada, New Zealand and European countries such as the United Kingdom, Germany, Denmark and Spain for the treatment of spasticity because of 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.

Taking into account the good therapeutic management of cannabinoids in cachexia and malnutrition associated with cancer and AIDS, it appears feasible that this kind of pharmacotherapy could also be 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 first involved 11 AN patients in a 4-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 that it was an inpatient study and occasional tube feeding was used. In addition, THC was compared with diazepam instead of placebo, which could be a confounding factor, given that diazepam has also been reported to increase food intake per se (Naruse et al., 1991). The second study involved nine AN outpatients treated with THC. The results showed a significant improvement in depression and perfectionism scores without improving weight gain (Berry, 2006).

Currently, there is an ongoing phase III clinical trial involving 22 patients to determine whether severe chronic AN patients treated with Marinol show significant weight improvement, with secondary objectives being the evaluation of the eating disorder inventory scale, motor and inner restlessness and endocrine (https://www.clinicaltrialsregister.eu; EudraCT parameters Number: 2007-005631-29). With this very limited number of trials (the last one being still in progress), it seems clear that no conclusions can be drawn on the therapeutic validity of a cannabinoid-based approach in eating disorders. However, the satisfactory clinical use of cannabinoid agonists in other pathologies requires further clinical trials on patients with eating disorders.

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 a stimulation of appetite is desired (Farrimond et al., 2012).

Conclusion

The eCB system is a lipid signalling system comprising all the molecular machinery required to properly activate the cannabinoid receptors. In terms of energy homoeostasis, 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 systems that can coordinate all the players involved in energy balance, thus being an interesting target in all the diseases related to an imbalanced energy homoeostasis such as 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 to feeding behaviour, especially those related to the reward system, where the eCB system plays 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, such as cachexia in AIDS patients; however, there have been very few trials on the therapeutic validity of cannabinoids in eating disorders and the results are inconclusive. Taken together, all these considerations should lead to the development of new clinical trials for assessing cannabinoid agonists in the management of eating disorders.

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Conflicts of interest

There are no conflicts of interest.

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