The role of the endocannabinoid system in the neuroendocrine regulation of energy balance

Francisco Javier Bermudez-Silva, Pierre Cardinal and Daniela Cota

J Psychopharmacol 2012 26: 114 originally published online 8 August 2011
DOI: 10.1177/0269881111408458

Review

The role of the endocannabinoid system in the neuroendocrine regulation of energy balance

Francisco Javier Bermudez-Silva[1,2,3,4], Pierre Cardinal[1,2] and Daniela Cota[1,2]

Journal of Psychopharmacology
26(1) 114–124

DOI: 10.1177/0269881111408458
jop.sagepub.com

Abstract

Animal and human studies carried out so far have established a role for the endocannabinoid system (ECS) in the regulation of energy balance. Here we critically discuss the role of the endocannabinoid signalling in brain structures, such as the hypothalamus and reward-related areas, and its interaction with neurotransmitter and neuropeptide systems involved in the regulation of food intake and body weight. The ECS has been found to interact with peripheral signals, like leptin, insulin, ghrelin and satiety hormones and the resulting effects on both central and peripheral mechanisms affecting energy balance and adiposity will be described. Furthermore, ECS dysregulation has been associated with the development of dyslipidemia, glucose intolerance and obesity; phenomena that are often accompanied by a plethora of neuroendocrine alterations which might play a causal role in determining ECS dysregulation.

Despite the withdrawal of the first generation of cannabinoid type 1 receptor (CB1) antagonists from the pharmaceutical market due to the occurrence of psychiatric adverse events, new evidence suggests that peripherally restricted CB1 antagonists might be efficacious for the treatment of obesity and its associated metabolic disorders. Thus, a perspective on new promising strategies to selectively target the ECS in the context of energy balance regulation is given.

Keywords

CB1, central nervous system, endocannabinoid, energy balance, food intake, hypothalamus, obesity, reward circuits

Introduction

The central nervous system (CNS) is critical for the coordination of the molecular, metabolic and behavioural mechanisms ensuring that the right nutrients get to the tissues when they need them. A complex and integrated neuronal network receives and integrates information about the type and quantity of nutrients recently ingested through the gastrointestinal tract, coordinates the use of energy substrates across various organs, monitors stored levels of energy in the adipose tissue and responds to external cues informing about food availability and palatability, thus regulating food intake.

Two major categories of signals that arise from the periphery have important effects on CNS circuits controlling energy balance. The first category is given by 'adiposity' signals, which include the hormones leptin and insulin, and that are able to inform the brain about levels of stored energy in the body (Morton et al., 2006). An important feature of adiposity signals is their ability to directly access key structures in the brain and therefore influence energy homeostasis. The second category includes satiety signals that consist of several hormones produced in the gastrointestinal tract, like cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), whose levels rise during the meal and help determine satiety and consequent meal termination (Strader and Woods, 2005). An exception to this category of signals is the gastric hormone ghrelin, which increases before meals and favours food intake and body weight gain (Wiedmer et al., 2007).

Recent evidence has also highlighted how nutrients, and particularly glucose, long-chain fatty acids and certain types of amino acids, might work as signals directly informing the CNS about the status of currently available energy (Cota et al., 2007; Lam et al., 2005; Xue and Kahn, 2006).

In addition, several neurotransmitters and neuropeptides contribute to energy balance regulation, by transferring

[1]INSERM U862, Avenir group 'Energy Balance and Obesity', Bordeaux, France.
[2]Université de Bordeaux 2, Bordeaux, France.
[3]Fundacion IMABIS, Hospital Carlos Haya de Malaga, Laboratorio de Medicina Regenerativa, Malaga, Spain.
[4]CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Santiago de Compostela, Spain.

Corresponding author:
Daniela Cota, INSERM U862, Avenir Group 'Energy Balance and Obesity', NeuroCentre François Magendie, Université de Bordeaux 2, 146 Rue Léo Saignat, 33077 Bordeaux, France
Email: daniela.cota@inserm.fr

information among different neuronal circuits. Some of these latter signals are derived from modified amino acids, like glutamate and γ-aminobutyric acid (GABA), or biogenic amines, like catecholamines, serotonin and acetylcholine. Others are constituted by simple chains of amino acids; while certain types of lipids, like steroids, have been long known to work as CNS signalling molecules. Among the lipid-derived molecules that work as intercellular signals able to affect energy balance regulation, we now also have to include the endocannabinoids, fatty acid derivatives that within the CNS work as local neuromodulators able to directly regulate rate of release of classical neurotransmitters.

The appetite-inducing effects of marijuana (*Cannabis sativa*) have been known for centuries. However, these effects were seriously taken into consideration in research only after the discovery of the endogenous cannabinoid system (ECS), which provided a physiological substrate for the action of marijuana and its derivatives. This system includes at least two cannabinoid receptors, specific endogenous ligands, called endocannabinoids, and their biosynthesis and degradation pathways (Di Marzo, 2009; Katona and Freund, 2008).

The first cannabinoid receptor or CB1 was identified and cloned after screening for the affinity of already characterized orphan G-protein coupled receptors to the main psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC) (Devane et al., 1988; Matsuda et al., 1990). The second cannabinoid receptor or CB2 was cloned soon thereafter (Munro et al., 1993). Both are metabotropic receptors coupled to G-proteins of the Gi/o type and their transduction systems include the modulation of several intracellular pathways (reviewed by Howlett and et al., 2002). Among the two receptors discovered so far, CB1 has been widely investigated for its role in energy balance regulation (Di Marzo et al., 2009a; Pagotto et al., 2006), emerging as a potential target for the treatment of obesity (Cota, 2007; Di Marzo, 2008).

CB1 is widely expressed in the brain, including areas associated with the regulation of energy homeostasis, like the hypothalamus, the brainstem and the cortico-limbic system, and is also present in metabolically relevant peripheral organs, such as the liver, pancreas, muscle and adipose tissue (Di Marzo et al., 2009a; Pagotto et al., 2006).

Endocannabinoids are polyunsaturated fatty acids derivatives. So far, at least 5 endocannabinoids have been identified, of which the N-ethanolamide of arachidonic acid, also known as anandamide, and the glyceryl ester of arachidonic acid or 2-arachidonoylglycerol (2-AG) are the best characterized. Anandamide and 2-AG biosynthesis involves cell membrane phospholipids and specific enzymes (Di Marzo, 2009; Katona and Freund, 2008). Within the CNS, endocannabinoids are released immediately after their synthesis, act on CB1 mainly located presynaptically and are thereafter immediately metabolized (Di Marzo, 2009; Katona and Freund, 2008). Their degradation requires cellular reuptake and enzymatic hydrolysis, which is under the control of a fatty acid amide hydrolase (FAAH) for anandamide, and a monoacylglycerol lipase (MAGL) for 2-AG (Di Marzo, 2009; Katona and Freund, 2008). Although a putative transporter has not been identified yet, there are several synthetic compounds that are considered inhibitors of endocannabinoid cellular reuptake (Di Marzo, 2009).

As briefly mentioned before, the ECS has attracted the attention of researchers working in the field of energy balance regulation, since synthetic compounds like the CB1 antagonist/inverse agonist rimonabant have shown potential for the treatment of diet-induced obesity. Indeed, chronic pharmacological blockade of CB1 in animal models of obesity and in humans not only decreases food intake and body weight, but also improves lipid metabolism and insulin sensitivity (Cota, 2007; Di Marzo and Despres, 2009). These observations are in agreement with data obtained from mice lacking CB1 ($CB1^{-/-}$), which clearly demonstrated that the ECS is an essential endogenous regulator of energy balance and peripheral metabolism, whose actions on food intake and body weight are dependent upon the functional expression and activity of CB1 (Cota et al., 2003). Unfortunately, the initial enthusiasm for the clinical use of rimonabant (commercial name Acomplia) faded away because of the important psychiatric side effects reported, which led to its withdrawal from the European market in January 2009 (European Medicines Agency, 2009). Nevertheless, it is clear that a better knowledge of the broad mode of action of the ECS will potentially allow targeting the system in a more selective and specific way, thus outweighing or reducing possible side effects. This review therefore provides an overview of the recent advancements made in understanding the role of this system in the neuroendocrine regulation of energy balance.

The ECS, hypothalamic circuits and energy balance regulation

Endocannabinoids and CB1 are produced and expressed in the hypothalamus (Bisogno et al., 1999; Herkenham et al., 1991), one of the main brain areas involved in the regulation of energy balance (Morton et al., 2006). Remarkably, although hypothalamic CB1 expression is among the lowest in the brain, activation of the receptors shows high efficiency and leads to profound effects (Breivogel and Childers, 1998).

First evidence of a role for the ECS in the hypothalamic control of food intake came from studies demonstrating that the systemic administration of Δ^9-THC facilitated eating elicited by electrical stimulation of the lateral hypothalamus (LH) (Trojniar and Wise, 1991). In fact, peripheral as well as intra-hypothalamic administration of endocannabinoids or CB1 agonists increases food intake in rodents, an effect that is prevented by pre-treating the animals with a CB1 antagonist (Cota, 2007). In addition, hypothalamic 2-AG levels are inversely correlated to the body's energy status, increasing during fasting and declining during refeeding (Kirkham et al., 2002).

It is now well established that the activation of arcuate nucleus (ARC) neurons expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) decreases food intake and body weight (Cone, 2005; Morton et al., 2006). Whereas, activation of ARC neurons co-expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP) increases food intake and body weight (Cone, 2005; Morton et al., 2006). Furthermore, these neuronal populations can easily sense hormonal and nutrient

signals coming from the periphery (Cota et al., 2007; Lam et al., 2005; Xue and Kahn, 2006).

POMC and AgRP produced in the ARC oppositely regulate the activity of melanocortin receptors located in the paraventricular nucleus (PVN), all together constituting the hypothalamic melanocortin system (Cone, 2005). CB1 is expressed on GABAergic terminals entering the ARC and POMC cells constitutively produce endocannabinoids, which inhibit GABA release onto POMC neurons (Hentges et al., 2005); an effect that is, however, present only on a distinct set of POMC dendrites that extend into preoptic hypothalamic regions (Hentges, 2007). The food intake effects of either CB1 agonists or antagonists do not require the melanocortin system; conversely the intracerebroventricular (icv) administration of a CB1 antagonist attenuates the orexigenic effect of melanocortin antagonists, suggesting that CB1 acts downstream from melanocortin receptors (Verty et al., 2004). Melanocortin agonists administered icv do not alter hypothalamic anandamide or 2-AG levels (Matias et al., 2008b). While central administration of melanocortin receptor antagonists produces a delayed increase in hypothalamic endocannabinoid levels (Matias et al., 2008b).

Using a rat hypothalamic explant model, it was also shown that anandamide increases NPY secretion, an effect dependent upon CB1 activation and consistent with the ability of endocannabinoids to stimulate food intake (Gamber et al., 2005). However, the CB1 antagonist rimonabant is equally active at reducing food intake in wild type and NPY null mice (Di Marzo et al., 2001), making it unlikely that endocannabinoids modulate food intake through NPY.

Within the PVN, glucocorticoids, which have a known orexigenic action, rapidly inhibit magnocellular (which produce vasopression and oxytocin) and parvocellular (which produce CRH and thyrotropin-releasing hormone) neurons by suppressing excitatory glutamatergic synaptic inputs through retrograde release of endocannabinoids and CB1 activation (Tasker, 2006). Thus, glucocorticoids use a fast-feedback mechanism relying on the activation of a specific glucocorticoid membrane receptor, which leads to the modulation of neuroendocrine responses involved in stress and energy balance regulation via the ECS (Tasker, 2006).

Orexins and melanin-concentrating hormone (MCH) neurons located in the LH have been also implicated in the regulation of energy balance (Morrison and Berthoud, 2007). In particular, orexins neurons might affect feeding behaviour by modulating arousal (Yamanaka et al., 2003). Interestingly, in vitro studies have shown that CB1 and orexin-1 receptors are present as heterodimers/oligomers in intracellular vesicles and that treatment with the CB1 antagonist rimonabant blocks the ability of orexin A to activate intracellular pathways under the control of the orexin receptor (Ellis et al., 2006). Furthermore, and similarly to what has already reported about glucocorticoids action in the PVN, the ability of both orexins and MCH to modulate neuronal excitability seems mediated by retrograde endocannabinoid release (Haj-Dahmane and Shen, 2005; Jo et al., 2005). Using patch-clamp recording, Huang et al. (2007) have recently found that CB1 agonists inhibit orexin neurons while exciting MCH neurons. These two opposite actions, which are CB1 dependent, are respectively due to the attenuation of presynaptic glutamate and GABA.

As a result, the effects of endocannabinoids as well as of CB1 agonists and antagonists on energy balance might be mediated by several of the neuronal circuits and neuropeptides described above. For instance, acute intraperitoneal administration of rimonabant (5 mg/kg) reduces food intake and induces c-fos (a marker of early neuronal activation) expression in several hypothalamic nuclei, including the ARC, the PVN and the LH (Verty et al., 2009b).

Figure 1 summarizes the functional relationship of the ECS, hypothalamic circuits and some peripheral signals participating in energy balance regulation.

Clearly, the hypothalamus is not the only CNS structure involved in such regulation. Information concerning the availability and variety of food is processed in cortico-limbic areas, which modulate responses to rewarding stimuli (Berridge et al., 2010; Morrison and Berthoud, 2007). The relationship between the ECS and the reward-related circuits will be briefly illustrated in the following section.

The ECS, reward circuits and energy balance regulation

The reward system able to process and integrate 'liking' (pleasure/palatability) and 'wanting' (appetite/incentive motivation) perceptions associated with the availability and variety of food, comprises a series of synaptically interconnected circuits linking the prefrontal cortex, the amygdala, the ventral tegmental area (VTA), the nucleus accumbens (NAc) and the ventral pallidum. This integrated network connects forebrain, hindbrain and midbrain areas with hypothalamic areas and is thus able to modulate feeding behaviour (Berridge et al., 2010).

Similar to what has already been reported for the hypothalamus, fasting raises endocannabinoid levels in the rat limbic forebrain structures, including the NAc (Kirkham et al., 2002). Furthermore, direct administration of 2-AG into the NAc shell, an area strongly linked to eating motivation, increases food intake in a CB1-dependent manner (Kirkham et al., 2002). Intriguingly, the NAc shell is the only cortico-striatal structure to send projections directly to the LH, thus suggesting its unique relationship with a hypothalamic area regulating food intake (Kelley et al., 2005). This relationship could be functionally mediated by the ECS. Administration of anandamide into the NAc shell in fact induces c-fos expression in several hypothalamic nuclei, including the LH, the dorsomedial nucleus and the PVN (Soria-Gomez et al., 2007).

Another possibility is that the increase in endocannabinoids within the NAc shell might enhance pleasure from food. Over time, several studies have pointed out the ability of endocannabinoids or CB1 agonists to increase consumption of palatable food, or conversely, the ability of CB1 antagonists to preferentially, although not exclusively, inhibit the intake of palatable food (Cota et al., 2006). Using a taste reactivity paradigm, Mahler et al. (2007) recently confirmed that the ECS modulates palatability, by reporting that anandamide microinjections into the medial part of the NAc shell doubled the number of 'liking' reactions to

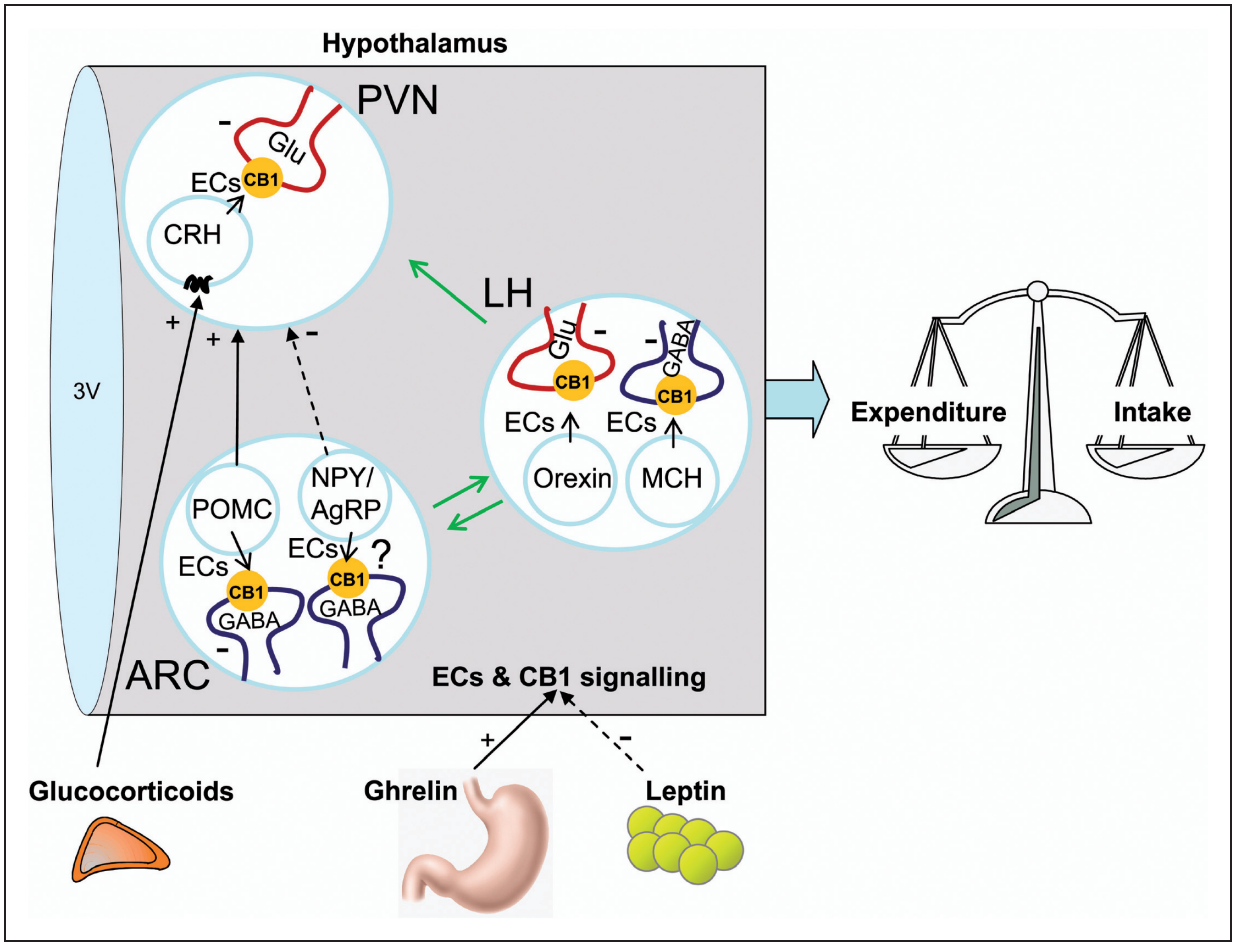

Figure 1. Functional relationship among the ECS, the neuroendocrine circuits and peripheral signals participating in the hypothalamic regulation of energy balance. Intra-hypothalamic arrows denote projection pathways; dashed lines denote inhibition. For didactic reasons some intra-hypothalamic neuronal populations and connections have been omitted. Please see the text for further detail. AgRP, agouti-related protein; ARC, arcuate nucleus; CB1, cannabinoid type 1 receptor; CRH, corticotropin-releasing hormone; ECs, endocannabinoids; GABA, γ-aminobutyric acid; Glu, glutamate; LH, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; POMC, pro-opio-melanocortin; PVN, paraventricular nucleus; 3 V, third ventricle.

intraoral sucrose in rats, without altering 'disliking' reactions to bitter quinine.

In the context of the role of the ECS in modulating liking responses to food, the interaction of the ECS with the endogenous opioid system should be highlighted. For instance, there is considerable anatomical overlap among CB1, opioid receptors and their respective endogenous ligands in brain areas regulating food intake and reward mechanisms (Cota et al., 2006). Additionally, the co-administration of subthreshold doses of naloxone and rimonabant suppresses food intake to a much greater extent than the effect obtained by the administration of each drug alone, thus pointing to a synergistic interaction between opioid and cannabinoid antagonists (Rowland et al., 2001).

Several studies have also implicated the ECS in the processes underlying the motivation to obtain food. This role can be in part explained taking into account the existing relationship between the ECS and the mesolimbic dopaminergic system.

Dopamine is considered a crucial mediator of the rewarding effects of food and drugs of abuse. Importantly, the dopamine release induced by the exposure to a novel highly palatable food (candied cherry) in the NAc shell is inhibited by the systemic administration of the CB1 antagonist rimonabant (Melis et al., 2007). Thus, exposure to palatable food might increase endocannabinoid levels in the NAc shell, an effect that in turn induces dopamine release in this brain area. Accordingly, chronic exposure to a high-fat, palatable diet decreases CB1 expression in the NAc shell, a phenomenon that could be interpreted as the resulting effect of increased endocannabinoid levels (Harrold et al., 2002). How the ECS might actually affect dopamine release in the NAc shell is, however, still matter of debate. A possibility is that endocannabinoids acting mainly on CB1 receptors on the axon terminals of glutamatergic neurons inhibit glutamate release, thus inhibiting the GABAergic neurons that originate in the NAc and project to the VTA, consequently disinhibiting VTA dopaminergic neurons (Maldonado et al., 2006). Besides,

endocannabinoids are synthesized by VTA dopaminergic neurons and participate to the fine-tuned regulation of this neuronal population. In fact, CB1 receptors are located on presynaptic glutamatergic and GABAergic neurons in the VTA, but not on dopaminergic neurons (Maldonado et al., 2006).

In summary, the ECS influences feeding behaviour by acting upon circuits located in both the hypothalamus and cortico-limbic structures, with an overall net anabolic effect. However, the role of the ECS in the CNS control of energy balance might be far more complicated than it appears to be. Bellocchio et al. (2010) have recently shown that the CB1-dependent control of glutamatergic or GABAergic transmission exerts opposite effects on short-term stimulated food intake. In particular, the CB1-dependent acute inhibition of excitatory glutamatergic transmission contributes to the well-known orexigenic effect of Δ^9-THC; while inhibition of inhibitory GABAergic transmission in the NAc mediates an unforeseen hypophagic effect of the endogenous CB1 signalling (Bellocchio et al., 2010).

Finally, both hypothalamic and reward-related circuits represent the target of the action of peripheral signals involved in the control of energy balance, such as leptin, ghrelin and satiety hormones. Their relationship with the ECS will be further discussed below.

The ECS and leptin

As briefly mentioned before, the adiposity signal leptin plays a critical role in energy balance regulation. In 2001, Di Marzo et al. first described that leptin inhibits endocannabinoid production in the hypothalamus (Di Marzo et al., 2001). Conversely, hypothalamic endocannabinoids are increased in genetically obese rodents lacking leptin or its receptor, and treatment of these mice with a CB1 antagonist attenuates their hyperphagia and retards their weight gain, implying that ECS over-activity may be a contributing factor in some animal models of genetic obesity (Di Marzo et al., 2001). More direct evidence linking endocannabinoids and leptin in the modulation of hypothalamic neuronal activity has come from electrophysiological studies. In fact, the CB1-dependent suppression of inhibition of orexigenic MCH neurons in the LH is blocked by leptin, which, by reducing intracellular calcium levels in MCH neurons, directly inhibits endocannabinoid synthesis (Jo et al., 2005). In the same fashion, leptin also blocks glucocorticoid-mediated endocannabinoid release in the PVN (Malcher-Lopes et al., 2006).

The relationship between the ECS and leptin in brain reward circuits is at present poorly investigated. However, it is known that leptin inhibits the mesolimbic dopamine system by acting on both the LH and the VTA (Hommel et al., 2006; Leinninger et al., 2009). Furthermore, obese rats with defective leptin signalling have increased CB1 expression and binding in reward brain structures, thus implying that the increased motivation to eat that characterizes this animal model might be due to a higher endocannabinoid tone in reward-related brain areas (Thanos et al., 2008).

Notably, the ECS-leptin interaction is not only used in a local way to integrate energy balance signals in the CNS but it also acts on distant processes occurring in other metabolically relevant organs, like the white adipose tissue. Indeed, administration of leptin in the medio-basal hypothalamus inhibits white adipose tissue lipogenesis by engaging hypothalamic phosphoinositide 3-kinase (PI3K) signalling, activating sympathetic innervations on the adipose tissue and locally inhibiting anandamide production (Buettner et al., 2008).

The ECS and satiety hormones

Gastrointestinal hormones like CCK, GLP-1 and PYY also exert important functions in the context of energy balance, by regulating meal duration and termination and inducing satiety (Strader and Woods, 2005). This function relies on the regulation of several neuroendocrine mechanisms, including the modulation of vagal afferents and the activation of neurons located in the nucleus of the solitary tract (NTS) in the brainstem (Strader and Woods, 2005).

Unfortunately, very little is known of the possible relationship between the ECS and satiety hormones. Recent studies by Burdyga et al. (2004) have however demonstrated that CB1 receptors expression in the vagal afferent neurons (VAN) is increased in fasting and decreased after refeeding. This decrease can be blocked by the administration of a CCK-1 receptor antagonist. Conversely, the fasting-induced increase in CB1 mRNA can be inhibited by peripheral CCK administration (Burdyga et al., 2004). Interestingly, the down-regulation of CB1 expression during refeeding is prevented by the administration of ghrelin, suggesting a role for ghrelin in blocking the CCK action on CB1 expression (Burdyga et al., 2006; Burdyga et al., 2010). Furthermore, the effects of CCK on CB1 expression can also be blocked by co-administration of anandamide, while the fasting-induced increase of CB1 mRNA can be prevented by using the CB1 antagonist AM281 (Burdyga et al., 2010). The latter finding thus implies the existence of an autoregulatory mechanism for CB1 expression in the vagus. Apart from CCK, there might also be a potential link between the ECS and PYY, since expression of Y2R, the receptor subtype mediating the anorectic action of PYY (Batterham et al., 2002), and CB1 mRNAs are oppositely regulated in the VAN (Burdyga et al., 2010). Y2R mRNA expression is increased by refeeding or CCK treatment, while high doses of anandamide are able to block CCK-induced increase of Y2R expression (Burdyga et al., 2010). To our knowledge, no link has been established so far between CB1 and GLP-1; the only paper that explored this issue found no modification of plasma GLP-1 levels following the administration of rimonabant in rats (Cani et al., 2004).

The ECS and ghrelin

Ghrelin is the only gut hormone with a known orexigenic action (Wiedmer et al., 2007). Because of the general similar function played by the ECS and ghrelin in the regulation of energy balance, their possible interaction has been the focus of intense research in recent years. As described in the previous section, ghrelin prevents refeeding-induced downregulation of CB1 expression in the VAN (Burdyga et al., 2006). However, the first evidence of cross-talk between ghrelin and

the ECS came in 2004, when a study by Tucci et al. showed that a sub-anorectic dose of rimonabant was able to abolish the orexigenic effect of an intra-PVN injection of ghrelin in rats, thus hinting that endocannabinoids might mediate ghrelin's action on food intake (Tucci et al., 2004). Indeed, later studies have demonstrated that ghrelin increases hypothalamic endocannabinoid content (Kola et al., 2008). Importantly, hypothalamic endocannabinoids might then participate in the activation of the AMP-activated protein kinase (AMPK), an intracellular fuel gauge known to mediate ghrelin's action within the hypothalamus (Andrews et al., 2008; Lopez et al., 2008).

On the other hand, systemic administration of the CB1 antagonist rimonabant reduces circulating ghrelin levels (Cani et al., 2004), suggesting that the release of ghrelin would be dependent on ECS activation. Indeed, the administration of CB1 agonists or methanandamide (an anandamide analog) in rats increases plasma ghrelin levels and ghrelin secretion from gastric X/A-like cells (Zbucki et al., 2008).

Therefore, taking this evidence into account, it could be proposed that endocannabinoids and ghrelin act synergistically in a feed-forward fashion, where each one would stimulate the release and the activity of the other to increase appetite and energy storage. In the periphery, endocannabinoids would favour ghrelin synthesis, whose release in the blood and subsequent action in the hypothalamus would in turn increase hypothalamic endocannabinoid content, activate CB1 and, by leading to an increase of hypothalamic AMPK activity, ultimately stimulate food intake and body weight gain.

The ECS in peripheral organs

Up to 2003, CB1 was thought to be exclusively located within the CNS. Work by Cota et al. and Bensaid et al. published in that year were the first to describe the functional presence of CB1 on white adipocytes (Bensaid et al., 2003; Cota et al., 2003). Furthermore, data obtained from zpair-feeding studies also implied that mechanisms other than the reduction in food intake might participate in the lean phenotype of $CB1^{-/-}$ mice (Cota et al., 2003). Since then, the role of the ECS in the regulation of lipid metabolism and use of substrates by peripheral organs has been substantiated. For instance, it has been shown that activation of CB1 receptors stimulates fat deposition by facilitating adipocyte differentiation and increasing expression of adipogenic enzymes and activity of the lipoprotein lipase (Bensaid et al., 2003; Cota et al., 2003; Matias et al., 2006; Muccioli et al., 2010). Interestingly, recent findings even suggest that the ECS might participate in the control of the transdifferentiation of the adipose tissue, since in vitro blockade of CB1 directly promotes transdifferentiation of white adipocytes into a mitochondria-rich, thermogenic brown fat phenotype (Perwitz et al., 2010). Accordingly, it has been reported that pharmacologic or genetic CB1 blockade increases mitochondrial biogenesis in white adipocytes via an endothelial nitric oxide synthase mechanism (Tedesco et al., 2008). These phenomena might in turn participate in the increase in energy expenditure, fatty acid oxidation and thermogenesis observed with the administration of CB1 antagonists in vivo (Cota et al., 2009; Herling et al., 2008; Nogueiras et al., 2008; Verty et al., 2009a). However, limiting the ability of the ECS to modulate energy expenditure solely by engaging peripheral, local mechanisms is over simplistic. In fact, it has been recently shown that mice selectively lacking CB1 expression in forebrain neurons and with a 60% reduction of the expression of the receptor in the cervical sympathetic ganglia have a lean phenotype and are resistant to diet-induced obesity (Quarta et al., 2010). This is due to an increase in lipid oxidation and thermogenesis caused by an enhanced sympathetic tone (Quarta et al., 2010).

Similarly to what was described for the white adipose tissue, the activation of CB1 in hepatocytes induces the expression of lipogenic enzymes, such as acetyl coenzyme-A carboxylase-1 (ACC1) and fatty acid synthase (FAS), which in turn increase de novo fatty acid synthesis and favour the development of liver steatosis, particularly during exposure to high-fat diets (Osei-Hyiaman et al., 2005; Osei-Hyiaman et al., 2008). The role of CB1 within the hepatocyte seems particularly important in the context of the regulation of lipid metabolism, since mice lacking the receptor in hepatocytes, although still susceptible to diet-induced obesity, are protected against liver steatosis, hyperglycemia, dyslipidemia and insulin resistance (Osei-Hyiaman et al., 2008).

Much less defined is the specific role of the ECS within the skeletal muscle and the endocrine pancreas. In isolated soleus muscle, pharmacological blockade of CB1 improves both basal and insulin-stimulated glucose transport activity, while CB1 activation has the opposite effect (Lindborg et al., 2010). Muscle cell cultures have also shown that the CB1 receptor can affect the responsiveness of skeletal muscle toward insulin through the modulation of the PI 3-kinase/PKB and the Raf-MEK1/2-ERK1/2 signalling pathways (Lipina et al., 2010).

Given the critical role of the endocrine pancreas in guaranteeing glucose homeostasis, a set of recent investigations has tried to uncover the function of the ECS in this organ. Several studies have demonstrated the presence of CB receptors (both CB1 and CB2) in rodent and human islets, with a species-dependent degree of expression (Li et al., 2011). The detailed physiological function of the ECS in the endocrine pancreas has not been delineated yet, and the reader should refer to reviews that have recently addressed this topic (Bermudez-Silva et al., 2009; Li et al., 2011; Nogueiras et al., 2009). Nevertheless, few reports indicate that CB receptors modulate insulin secretion by regulating glucose-induced calcium transients (Bermudez-Silva et al., 2009; Li et al., 2011; Nogueiras et al., 2009). Whether this local action might then have a relevant role in the maintenance of the glucose homeostasis in vivo is not known.

Finally, apart from the relationship between the ECS and gastrointestinal hormones that we have illustrated in previous sections, and the ECS ability to regulate gastrointestinal motility and secretion (Storr and Sharkey, 2007), recent evidence suggests that endocannabinoid signalling might link gut microbiota to the regulation of adipogenesis (Muccioli et al., 2010). Gut microbiota are known to influence whole-body metabolism and energy balance (Turnbaugh et al., 2006). Muccioli et al. have demonstrated that gut microbiota control the intestinal ECS tone, which in turn modulates gut -permeability (Muccioli et al., 2010). In fact, CB1

stimulation in a colonic epithelial monolayer cell model increases permeability. This is associated with increased lipopolysaccharide levels, which exacerbate gut permeability and ECS tone in both the gastrointestinal tract and the white adipose tissue (Muccioli et al., 2010). In obesity, the ECS is dysregulated (see also the following section); such dysregulation at the level of the gatstrointestinal tract and adipose tissue might therefore participate in the metabolic impairments which characterize the obese condition.

Altogether, it is clear that the understanding of the ECS role in metabolically relevant peripheral tissues has greatly advanced during the past few years. Presently, however, the relative contribution of CNS versus peripheral ECS in the modulation of energy balance is still a matter of debate. As will be further detailed below, peripherally restricted CB1 antagonists show hope for the treatment of obesity. An important next step will be therefore to exactly define their mechanisms of action.

The ECS, neuroendocrine dysregulation and obesity

Obesity is characterized by the accumulation of excess body fat, to a point that it is harmful for health. This condition is associated with a plethora of neuroendocrine alterations of which the most representative are the development of leptin and insulin resistance at both peripheral and central level. Thus, the ECS dysregulation extensively described in both obese humans and animals in recent years (Di Marzo and Despres, 2009) could be the result of neuroendocrine alterations causally linked to hyperphagia, overweight and an expanding waist.

For instance, it is important to mention again here that genetic defects in leptin production or signalling lead to increased endocannabinoid levels, particularly in the hypothalamus (Di Marzo et al., 2001) and to increased CB1 binding in reward brain structures like the NAc (Thanos et al., 2008). Moreover, it has been proposed that endocannabinoid fluctuations in reward-related brain areas could modulate the hedonic aspects of eating and might be elevated in obesity (Di Marzo et al., 2009a). However, the presence and function of an up-regulated ECS in brain reward circuits still deserves further investigation. Complex and still under-investigated is also the relationship between the action of endocrine signals within the CNS and the modulation of the peripheral ECS. In this context, it has been shown that intra-hypothalamic leptin fails to suppress white adipose tissue anandamide levels and lipogenesis when a CB1 agonist is systemically administered (Buettner et al., 2008). Thus, the failure of leptin-driven pathways to restrain endocannabinoid levels in the adipose tissue might contribute to the ECS over-activity observed in obesity (Buettner et al., 2008).

While leptin resistance seems to have a key role in endocannabinoid up-regulation in the hypothalamus and white adipose tissue, insulin resistance is emerging as one of the most important events related with the up-regulated peripheral endocannabinoid tone observed in obesity (Di Marzo et al., 2009b; Matias et al., 2006; Murdolo et al., 2007). In fact, a recent study carried out in humans suggests that insulin negatively regulates circulating endocannabinoids and, in particular, anandamide levels (Di Marzo et al., 2009b). This phenomenon is inversely related to metabolic predictors of insulin resistance and dyslipidemia (Di Marzo et al., 2009b).

In addition, the decreased release of satiety hormones, such as PYY, which is normally found in obesity (le Roux et al., 2006) might also favour the maintenance of ECS activation in the gastro-intestinal tract, which in turn could sustain further food intake. Interestingly, obese Zucker rats (a model of genetic obesity characterized by important hyperphagia) not only have increased anandamide and 2-AG levels in the duodenum as compared with lean animals, but they also show an altered modulation of these endocannabinoids in response to fasting/refeeding, and in particular they remain elevated even after refeeding (Izzo et al., 2009).

Alternatively, there is also evidence pointing to a direct over-activity of the ECS, which could causally lead to obesity.

Chronic overconsumption of high-fat diets causes increased availability of polyunsaturated fatty acid precursors for the synthesis of endocannabinoids (Berger et al., 2001). Moreover, although increased endocannabinoid levels have been found in several peripheral tissues and endocrine organs, their onset, duration and extent of the increase closely depend on the fatty acid composition of the diet (Matias et al., 2008a). Under this scenario, the increased endocannabinoid levels derived from the diet would be maintained by the obesity-induced leptin and insulin resistance, thus leading to a chronically elevated endocannabinoid tone, able to reinforce the hedonic properties of the food and favouring further food intake (Di Marzo and Despres, 2009).

Additionally, genetic studies have been carried out assessing CNR1 (coding for CB1 receptor) and FAAH polymorphisms in obese populations. Despite some conflicting results, associations between polymorphisms in these genes and abdominal obesity (Sipe et al., 2005), dyslipidemia (Baye et al., 2008) or metabolic syndrome (Bordicchia et al., 2010) have been described, which however suggest only a partial and weak contribution to the obese phenotype. Possibly, this genetic variation may influence obesity depending on the diet (Aberle et al., 2008).

Independently from the reasons that lead to ECS over-activity, and given that the ECS classically fine-tunes other systems and pathways in response to environmental stimuli, its dysregulation could be interpreted as the result of a local inappropriate cell functioning caused by upstream molecular events, which the ECS tries to compensate for.

Importantly, pharmacological antagonism of CB1 receptors decreases body weight and improves key metabolic parameters in both obese patients and animals (Cota, 2007; Di Marzo and Despres, 2009), thus implying that at least a dysregulated CB1 signalling is causally involved in the vicious cycle underlying obesity and its related metabolic consequences.

The ECS as an anti-obesity therapy target

Shortly after the discovery of cannabinoid receptors, specific CB1 antagonists were developed. The first of these drugs was SR141716A, also known as rimonabant (Rinaldi-Carmona

et al., 1994). Chronic administration of this compound is effective in reducing body weight and fat mass, while improving glucose homeostasis, insulin sensitivity and overall cardiometabolic risk profile in obese rodents and humans (Cota, 2007; Di Marzo and Despres, 2009). However, concern over neuropsychiatric side effects, including anxiety, depression, and suicidal ideation, as well as probing editorials and critical commentaries pointing to the use of non-validated or disputed surrogate endpoints and lack of generalizability characterizing the clinical trials testing rimonabant in obese populations prevented its approval in the United States and led to its withdrawal from the European market (Jones, 2008).

A great controversy in both the research community and the pharmaceutical industry has then followed about the therapeutic use of CB1 antagonists in obesity and related metabolic disorders (Bermudez-Silva et al., 2010; Di Marzo and Despres, 2009).

However, it should be pointed out once more that the ECS is strategically positioned in all key points modulating energy homeostasis and is able to directly affect relevant metabolic functions in peripheral organs. Taking this evidence into account, a new class of CB1 antagonists with decreased ability to pass the blood brain barrier is being explored as a potential new tool to tackle obesity. In a recent paper by Tam et al. it was shown that a new CB1 neutral antagonist largely restricted to the periphery does not affect behavioural responses mediated by CB1 in the brain, but it causes weight-independent improvements in glucose homeostasis, fatty liver, and plasma lipid profile in mice with genetic or diet-induced obesity (Tam et al., 2010).

In addition to the CB1 antagonism approach there is potential for other ECS-based therapy against obesity. As discussed above, obesity is characterized by an up-regulated ECS. Thus, pharmacological modulation of endocannabinoid levels in obesity might provide a more physiological approach than blocking CB1. In fact, CB1 blockade may induce a feedback loop driving endocannabinoid levels even higher, consequently modulating the activity of other receptors, such as CB2 and TRPV1 or the putative cannabinoid receptor GPR55. The effects of this crossover activation cannot be predicted. However, drugs targeting the enzymes responsible for the synthesis and degradation of endocannabinoids could be useful in counteracting the ECS over-activity. An example of this kind of drug is the compound O-5596, a diacylglicerol lipase inhibitor able to decrease endocannabinoid synthesis and inhibit food intake (Bisogno et al., 2009). Another putative strategy for reducing endocannabinoid over-activity could be by decreasing the availability of endocannabinoid precursors. This possibility could indeed deserve further investigation, since a diet enriched in (n-3) long-chain polyunsaturated fatty acids (PUFA) has been shown to decrease endocannabinoid levels in the visceral adipose tissue, liver and heart (Batetta et al., 2009).

Finally, given the tight control of energy balance, an effective therapy against obesity could rely on an adjunctive strategy. This type of therapeutic approach could for instance exploit the synergism between targeting an ECS-component and other systems involved in the regulation of food intake and body weight.

Concluding remarks

Animal and human studies carried out so far have clearly established a role for the ECS in the regulation of energy balance. Evidence has also been accumulated pointing to the existence of a functional relationship between the ECS and neuroendocrine mechanisms participating to the control of nutrient intake, metabolism and storage. However, further research is needed in order to clearly unravel the molecular mechanisms underlying the functional cross-talk between endocannabinoids and neuroendocrine signals in a tissue- and circuit-specific manner, thus potentially helping determine the causes leading to ECS dysregulation in obesity and the role played by this system in the pathophysiology of this disease.

Growing evidence suggests that pharmacological inhibition of CB1 might prove beneficial in tackling obesity and associated cardio-metabolic risk factors. Although first generation CB1 antagonists have proven to have an unsatisfactory safety profile, a new class of compounds preferentially targeting the ECS in the periphery might represent interesting pharmacologic options for the treatment of metabolic disorders in the near future. Their impact on the aforementioned signals participating in the regulation of energy balance is at present unknown.

Funding

This work was supported by Avenir INSERM and EFSD-Sanofi Aventis grants (DC), INSERM/Aquitaine Region PhD Fellowship Programme (PC), the Instituto de Salud Carlos III, Ministerio de Ciencia e Innovacion, Spain, grant 07/0880 (FJBS) and Consejería de Salud de la Junta de Andalucía, grant PI-0232/2008 (FJBS). FJBS has a research contract with the National System of Health (Instituto de Salud Carlos III; CP07/00283). FJBS has also received a BAE from Instituto de Salud Carlos III (BA09/90066).

Conflict of interest

None declared.

References

Aberle J, Flitsch J, Beck NA, et al. (2008) Genetic variation may influence obesity only under conditions of diet: analysis of three candidate genes. *Mol Genet Metab* 95: 188–191.

Andrews ZB, Liu ZW, Walllingford N, et al. (2008) UCP2 mediates ghrelin's action on NPY/AgRP neurons by lowering free radicals. *Nature* 454: 846–851.

Batetta B, Griinari M, Carta G, et al. (2009) Endocannabinoids may mediate the ability of (n-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker rats. *J Nutr* 139: 1495–1501.

Batterham RL, Cowley MA, Small CJ, et al. (2002) Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 418: 650–654.

Baye TM, Zhang Y, Smith E, et al. (2008) Genetic variation in cannabinoid receptor 1 (CNR1) is associated with derangements in lipid homeostasis, independent of body mass index. *Pharmacogenomics* 9: 1647–1656.

Bellocchio L, Lafenetre P, Cannich A, et al. (2010) Bimodal control of stimulated food intake by the endocannabinoid system. *Nat Neurosci* 13: 281–283.

Bensaid M, Gary-Bobo M, Esclangon A, et al. (2003) The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 63: 908–914.

Berger A, Crozier G, Bisogno T, et al. (2001) Anandamide and diet: inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding N-acylethanolamines in piglets. *Proc Natl Acad Sci USA* 98: 6402–6406.

Bermudez-Silva FJ, Suarez Perez J, Nadal A, et al. (2009) The role of the pancreatic endocannabinoid system in glucose metabolism. *Best Pract Res Clin Endocrinol Metab* 23: 87–102.

Bermudez-Silva FJ, Viveros MP, McPartland JM, et al. (2010) The endocannabinoid system, eating behavior and energy homeostasis: the end or a new beginning? *Pharmacol Biochem Behav* 95: 375–382.

Berridge KC, Ho CY, Richard JM, et al. (2010) The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. *Brain Res* 1350: 43–64.

Bisogno T, Berrendero F, Ambrosino G, et al. (1999) Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem Biophys Res Commun* 256: 377–380.

Bisogno T, Burston JJ, Rai R, et al. (2009) Synthesis and pharmacological activity of a potent inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol. *ChemMedChem* 4: 946–950.

Bordicchia M, Battistoni I, Mancinelli L, et al. (2010) Cannabinoid CB1 receptor expression in relation to visceral adipose depots, endocannabinoid levels, microvascular damage, and the presence of the Cnr1 A3813G variant in humans. *Metabolism* 59: 734–741.

Breivogel CS and Childers SR (1998) The functional neuroanatomy of brain cannabinoid receptors. *Neurobiol Dis* 5: 417–431.

Buettner C, Muse ED, Cheng A, et al. (2008) Leptin controls adipose tissue lipogenesis via central, STAT3-independent mechanisms. *Nat Med* 14: 667–675.

Burdyga G, Lal S, Varro A, et al. (2004) Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. *J Neurosci* 24: 2708–2715.

Burdyga G, Varro A, Dimaline R, et al. (2006) Ghrelin receptors in rat and human nodose ganglia: putative role in regulating CB-1 and MCH receptor abundance. *Am J Physiol Gastrointest Liver Physiol* 290: G1289–G1297.

Burdyga G, Varro A, Dimaline R, et al. (2010) Expression of cannabinoid CB1 receptors by vagal afferent neurons: kinetics and role in influencing neurochemical phenotype. *Am J Physiol Gastrointest Liver Physiol* 299: G63–G69.

Cani PD, Montoya ML, Neyrinck AM, et al. (2004) Potential modulation of plasma ghrelin and glucagon-like peptide-1 by anorexigenic cannabinoid compounds, SR141716A (rimonabant) and oleoylethanolamide. *Br J Nutr* 92: 757–761.

Cone RD (2005) Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 8: 571–578.

Cota D (2007) CB1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. *Diabetes Metab Res Rev* 23: 507–517.

Cota D, Marsicano G, Tschop M, et al. (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 112: 423–431.

Cota D, Proulx K and Seeley RJ (2007) The role of CNS fuel sensing in energy and glucose regulation. *Gastroenterology* 132: 2158–2168.

Cota D, Sandoval DA, Olivieri M, et al. (2009) Food Intake-independent Effects of CB1 Antagonism on Glucose and Lipid Metabolism. *Obesity (Silver Spring)* 17: 1641–1645.

Cota D, Tschop MH, Horvath TL, et al. (2006) Cannabinoids, opioids and eating behavior: the molecular face of hedonism? *Brain Res Rev* 51: 85–107.

Devane WA, Dysarz FAIII, Johnson MR, et al. (1988) Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34: 605–613.

Di Marzo V (2008) Targeting the endocannabinoid system: to enhance or reduce? *Nat Rev Drug Discov* 7: 438–455.

Di Marzo V (2009) The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol Res* 60: 77–84.

Di Marzo V and Despres JP (2009) CB1 antagonists for obesity–what lessons have we learned from rimonabant? *Nat Rev Endocrinol* 5: 633–638.

Di Marzo V, Goparaju SK, Wang L, et al. (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410: 822–825.

Di Marzo V, Ligresti A and Cristino L (2009a) The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. *Int J Obes (Lond)* 33 Suppl 2: S18–S24.

Di Marzo V, Verrijken A, Hakkarainen A, et al. (2009b) Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. *Eur J Endocrinol* 161: 715–722.

Ellis J, Pediani JD, Canals M, et al. (2006) Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *J Biol Chem* 281: 38812–38824.

European Medicines Agency (2009) European public assessment report (EPAR). Acomplia EPAR summary for the public. Retrieved from http://www.ema.europa.eu/humandocs/Humans/EPAR/acomplia/acomplia.htm (accessed 20 September 2010).

Gamber KM, Macarthur H and Westfall TC (2005) Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. *Neuropharmacology* 49: 646–652.

Haj-Dahmane S and Shen RY (2005) The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus serotonin neurons through retrograde endocannabinoid signaling. *J Neurosci* 25: 896–905.

Harrold JA, Elliott JC, King PJ, et al. (2002) Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: a role for endogenous cannabinoids in driving appetite for palatable food? *Brain Res* 952: 232–238.

Hentges ST (2007) Synaptic regulation of proopiomelanocortin neurons can occur distal to the arcuate nucleus. *J Neurophysiol* 97: 3298–3304.

Hentges ST, Low MJ and Williams JT (2005) Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. *J Neurosci* 25: 9746–9751.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR and Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11: 563–583.

Herling AW, Kilp S, Elvert R, et al. (2008) Increased energy expenditure contributes more to the body weight-reducing effect of rimonabant than reduced food intake in candy-fed wistar rats. *Endocrinology* 149: 2557–2566.

Hommel JD, Trinko R, Sears RM, et al. (2006) Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron* 51: 801–810.

Howlett AC, Barth F, Bonner TI, et al. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54: 161–202.

Huang H, Acuna-Goycolea C, Li Y, et al. (2007) Cannabinoids excite hypothalamic melanin-concentrating hormone but inhibit

hypocretin/orexin neurons: implications for cannabinoid actions on food intake and cognitive arousal. *J Neurosci* 27: 4870–4881.
Izzo AA, Piscitelli F, Capasso R, et al. (2009) Peripheral endocannabinoid dysregulation in obesity: relation to intestinal motility and energy processing induced by food deprivation and re-feeding. *Br J Pharmacol* 158: 451–461.
Jo YH, Chen YJ, Chua SC Jr, et al. (2005) Integration of endocannabinoid and leptin signaling in an appetite-related neural circuit. *Neuron* 48: 1055–1066.
Jones D (2008) End of the line for cannabinoid receptor 1 as an anti-obesity target? *Nat Rev Drug Discov* 7: 961–962.
Katona I and Freund TF (2008) Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* 14: 923–930.
Kelley AE, Baldo BA, Pratt WE, et al. (2005) Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav* 86: 773–795.
Kirkham TC, Williams CM, Fezza F, et al. (2002) Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol* 136: 550–557.
Kola B, Farkas I, Christ-Crain M, et al. (2008) The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS One* 3: e1797.
Lam TK, Schwartz GJ and Rossetti L (2005) Hypothalamic sensing of fatty acids. *Nat Neurosci* 8: 579–584.
le Roux CW, Batterham RL, Aylwin SJ, et al. (2006) Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology* 147: 3–8.
Leinninger GM, Jo YH, Leshan RL, et al. (2009) Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab* 10: 89–98.
Li C, Jones PM and Persaud SJ (2011) Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacol Ther* 129: 307–320.
Lindborg KA, Teachey MK, Jacob S, et al. (2010) Effects of in vitro antagonism of endocannabinoid-1 receptors on the glucose transport system in normal and insulin-resistant rat skeletal muscle. *Diabetes Obes Metab* 12: 722–730.
Lipina C, Stretton C, Hastings S, et al. (2010) Regulation of MAP kinase-directed mitogenic and protein kinase B-mediated signaling by cannabinoid receptor type 1 in skeletal muscle cells. *Diabetes* 59: 375–385.
Lopez M, Lage R, Saha AK, et al. (2008) Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. *Cell Metab* 7: 389–399.
Mahler SV, Smith KS and Berridge KC (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology* 32: 2267–2278.
Malcher-Lopes R, Di S, Marcheselli VS, et al. (2006) Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci* 26: 6643–6650.
Maldonado R, Valverde O and Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* 29: 225–232.
Matias I, Gonthier MP, Orlando P, et al. (2006) Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 91: 3171–3180.
Matias I, Petrosino S, Racioppi A, et al. (2008a) Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: Effect of high fat diets. *Mol Cell Endocrinol* 286: S66–S78.
Matias I, Vergoni AV, Petrosino S, et al. (2008b) Regulation of hypothalamic endocannabinoid levels by neuropeptides and hormones involved in food intake and metabolism: insulin and melanocortins. *Neuropharmacology* 54: 206–212.
Matsuda LA, Lolait SJ, Brownstein MJ, et al. (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561–564.
Melis T, Succu S, Sanna F, et al. (2007) The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. *Neurosci Lett* 419: 231–235.
Morrison CD and Berthoud HR (2007) Neurobiology of nutrition and obesity. *Nutr Rev* 65: 517–534.
Morton GJ, Cummings DE, Baskin DG, et al. (2006) Central nervous system control of food intake and body weight. *Nature* 443: 289–295.
Muccioli GG, Naslain D, Backhed F, et al. (2010) The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* 6: 392.
Munro S, Thomas KL and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61–65.
Murdolo G, Kempf K, Hammarstedt A, et al. (2007) Insulin differentially modulates the peripheral endocannabinoid system in human subcutaneous abdominal adipose tissue from lean and obese individuals. *J Endocrinol Invest* 30: RC17–RC21.
Nogueiras R, Diaz-Arteaga A, Lockie SH, et al. (2009) The endocannabinoid system: role in glucose and energy metabolism. *Pharmacol Res* 60: 93–98.
Nogueiras R, Veyrat-Durebex C, Suchanek PM, et al. (2008) Peripheral, but not central, CB1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. *Diabetes* 57: 2977–2991.
Osei-Hyiaman D, DePetrillo M, Pacher P, et al. (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 115: 1298–1305.
Osei-Hyiaman D, Liu J, Zhou L, et al. (2008) Hepatic CB1 receptor is required for development of diet-induced steatosis, dyslipidemia, and insulin and leptin resistance in mice. *J Clin Invest* 118: 3160–3169.
Pagotto U, Marsicano G, Cota D, et al. (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 27: 73–100.
Perwitz N, Wenzel J, Wagner I, et al. (2010) Cannabinoid type 1 receptor blockade induces transdifferentiation towards a brown fat phenotype in white adipocytes. *Diabetes Obes Metab* 12: 158–166.
Quarta C, Bellocchio L, Mancini G, et al. (2010) CB(1) signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. *Cell Metab* 11: 273–285.
Rinaldi-Carmona M, Barth F, Heaulme M, et al. (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350: 240–244.
Rowland NE, Mukherjee M and Robertson K (2001) Effects of the cannabinoid receptor antagonist SR 141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. *Psychopharmacology (Berl)* 159: 111–116.
Sipe JC, Waalen J, Gerber A, et al. (2005) Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). *Int J Obes (Lond)* 29: 755–759.
Soria-Gomez E, Matias I, Rueda-Orozco PE, et al. (2007) Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus. *Br J Pharmacol* 151: 1109–1116.

Storr MA and Sharkey KA (2007) The endocannabinoid system and gut-brain signalling. *Curr Opin Pharmacol* 7: 575–582.

Strader AD and Woods SC (2005) Gastrointestinal hormones and food intake. *Gastroenterology* 128: 175–191.

Tam J, Vemuri VK, Liu J, et al. (2010) Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest* 120: 2953–2966.

Tasker JG (2006) Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration. *Obesity (Silver Spring)* 14 Suppl 5: S259–S265.

Tedesco L, Valerio A, Cervino C, et al. (2008) Cannabinoid type 1 receptor blockade promotes mitochondrial biogenesis through endothelial nitric oxide synthase expression in white adipocytes. *Diabetes* 57: 2028–2036.

Thanos PK, Ramalhete RC, Michaelides M, et al. (2008) Leptin receptor deficiency is associated with upregulation of cannabinoid 1 receptors in limbic brain regions. *Synapse* 62: 637–642.

Trojniar W and Wise RA (1991) Facilitory effect of delta 9-tetrahydrocannabinol on hypothalamically induced feeding. *Psychopharmacology (Berl)* 103: 172–176.

Tucci SA, Rogers EK, Korbonits M, et al. (2004) The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. *Br J Pharmacol* 143: 520–523.

Turnbaugh PJ, Ley RE, Mahowald MA, et al. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444: 1027–1031.

Verty AN, Allen AM and Oldfield BJ (2009a) The effects of rimonabant on brown adipose tissue in rat: implications for energy expenditure. *Obesity (Silver Spring)* 17: 254–261.

Verty AN, Boon WM, Mallet PE, et al. (2009b) Involvement of hypothalamic peptides in the anorectic action of the CB receptor antagonist rimonabant (SR 141716). *Eur J Neurosci* 29: 2207–2216.

Verty AN, McFarlane JR, McGregor IS, et al. (2004) Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. *Endocrinology* 145: 3224–3231.

Wiedmer P, Nogueiras R, Broglio F, et al. (2007) Ghrelin, obesity and diabetes. *Nat Clin Pract Endocrinol Metab* 3: 705–712.

Xue B and Kahn BB (2006) AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. *J Physiol* 574: 73–83.

Yamanaka A, Beuckmann CT, Willie JT, et al. (2003) Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38: 701–713.

Zbucki RL, Sawicki B, Hryniewicz A, et al. (2008) Cannabinoids enhance gastric X/A-like cells activity. *Folia Histochem Cytobiol* 46: 219–224.