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REVIEW

New insights on the role of the endocannabinoid system in the regulation of energy balance

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Within the past 15 years, the endocannabinoid system (ECS) has emerged as a lipid signaling system critically involved in the regulation of energy balance, as it exerts a regulatory control on every aspect related to the search, the intake, the metabolism and the storage of calories. An overactive endocannabinoid cannabinoid type 1 (CB_1) receptor signaling promotes the development of obesity, insulin resistance and dyslipidemia, representing a valuable pharmacotherapeutic target for obesity and metabolic disorders. However, because of the psychiatric side effects, the first generation of brain-penetrant CB_1 receptor blockers developed as antiobesity treatment were removed from the European market in late 2008. Since then, recent studies have identified new mechanisms of action of the ECS in energy balance and metabolism, as well as novel ways of targeting the system that may be efficacious for the treatment of obesity and metabolic disorders. These aspects will be especially highlighted in this review.

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INTRODUCTION

The endocannabinoid system (ECS) monitors energy needs and metabolic responses in mammals by exerting a positive, anabolic control essentially on every aspect related to the intake and storage of calories. Accordingly, overactivity of the ECS is a characteristic feature of obesity and metabolic disorders in animals and humans.[1–4]

This system, which is found in most mammalian cells and tissues, encompasses specific endogenous ligands, called endocannabinoids, their biosynthesis and degradation pathways and at least two specific receptor types named cannabinoid type 1 (CB_1) and cannabinoid type 2 (CB_2).[5,6] Both CB_1 and CB_2 are metabotropic receptors coupled to G proteins of the Gi/o type and their transduction systems include the modulation of ionic channels and of several intracellular pathways, such as the adenylate cyclase and the mitogen-activated protein kinase pathways.[7] The CB_1 receptor is highly expressed throughout the central nervous system (CNS), including in neurons that regulate food intake, energy expenditure and reward-related responses, as well as in peripheral organs, such as liver, pancreas, muscle and adipose tissue.[4,8] Consequently, the CB_1 receptor has been widely investigated as a target for the treatment of obesity and metabolic disorders.[2,9] Differently from CB_1, the CB_2 receptor is mainly found in immune cells and participate to the regulation of immune and inflammatory responses.[7]

Endocannabinoids are polyunsaturated fatty acid (PUFA) derivatives generated on demand from cell membrane phospholipid precursors (Figure 1) that then act in an autocrine or paracrine manner on cannabinoid receptors.[5,6] The best characterized endocannabinoids are *N*-ethanolamide of arachidonic acid, also known as anandamide (AEA), and the glyceryl ester of arachidonic acid or 2-arachidonoylglycerol (2-AG) that are present in both central and peripheral neurons as well as in various types of parenchymal cells. Within the CNS, endocannabinoids classically work as retrograde neuromodulators, acting on CB_1 receptors mainly located presynaptically and leading to the suppression of neurotransmitter release.[10] Degradation of AEA and 2-AG requires their cellular reuptake and hydrolysis that is under the control of a fatty acid amide hydrolase for AEA and a monoacylglycerol lipase for 2-AG (Figure 1).[5,6]

Initial findings demonstrating that pharmacological blockade of CB_1 receptors inhibited food intake in rodents[11–13] provided the impetus for testing CB_1 receptor antagonists for the treatment of obesity. Indeed, chronic pharmacological blockade of CB_1 in obese animal models and in obese patients decreases food intake and body weight, while improving lipid and glucose metabolism.[2,14–18] Accordingly, mice lacking CB_1 are lean and resistant to diet-induced obesity.[19,20] Unfortunately, the important psychiatric side effects of rimonabant, the first CB_1 receptor antagonist approved for the treatment of obesity in several European countries, led to its withdrawal in late 2008. This event profoundly affected the pharmaceutical industry, with the discontinuation of development of all CB_1 receptor antagonists that at the time were under clinical/preclinical investigation. However, it also stimulated new investigations into the mechanisms of action of the ECS in energy balance and metabolism, with the aim of generating new knowledge that would allow targeting this system in a more specific and selective manner. Since then, recent studies have identified a role for the ECS in the modulation of taste and olfaction, which critically affect feeding behavior, and in the regulation of fat intake and preference, and other investigations have detailed some of the CNS circuits engaged to regulate peripheral metabolism and the important function played by the peripheral ECS especially in the regulation of insulin sensitivity. In addition, the characterization of novel CB_1 receptor antagonists that do not cause the well-known central side effects observed with rimonabant and the use of dietary strategies directly

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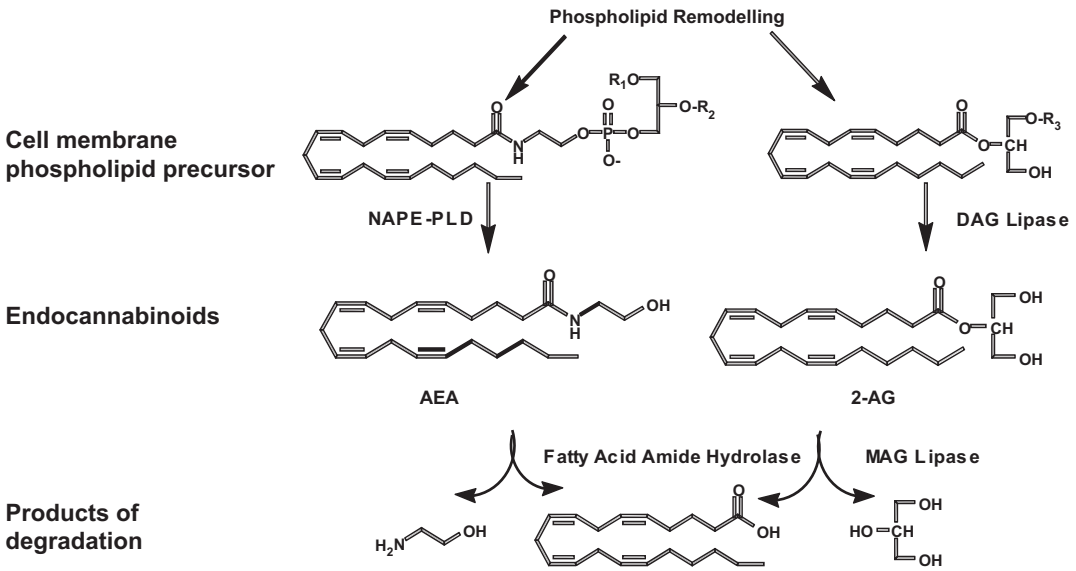

Figure 1. Schematic representation of the synthesis and degradation pathways of the two main endocannabinoids AEA and 2-AG. DAG, diacylglycerol; MAG, monoacylglycerol; NAPE-PLD, *N*-acyl phosphatidylethanolamine phospholipase D.

affecting endocannabinoid levels have reignited the interest around the ECS as therapeutic target.

Here we give an overview of the roles of the ECS in energy balance, discussing these latest advances and pinpointing the renewed interest of the field in this system and its therapeutic potential for the treatment of obesity and metabolic disorders.

SEARCH STRATEGY

A PubMed search of publications in English was conducted through the first week of December 2014. The following input was used for the search: cannabinoid AND (energy balance OR food intake OR obesity OR diabetes OR metabolic syndrome OR hypothalamus OR reward OR gut OR adipose tissue OR pancreas OR muscle OR liver OR diet). This search yielded more than 3500 potentially relevant articles. Additional articles were identified using the references listed in some of these articles. A total of 120 articles, the maximum allowed by the journal, were selected by the authors, who preferred recently published studies as well as articles that in the opinion of the authors were most significant and provided relevant information on the function of the ECS in the context of obesity and metabolic disease.

THE ECS AND THE CNS REGULATION OF ENERGY BALANCE AND METABOLISM

The CNS coordinates the molecular, metabolic and behavioral mechanisms that guarantee that the different tissues get the nutrients they need when they need them. Within the CNS, the endocannabinoids act as retrograde neuromodulators able to inhibit both excitatory and inhibitory neurotransmission by binding on presynaptic CB_1 receptors.[10] Thus, CB_1 receptor signaling plays a key role in modulating neuronal activity, particularly in brain areas participating to the regulation of energy balance, such as the hypothalamus,[21] the corticolimbic circuits,[22] including the nucleus accumbens (NAc) and the ventral tegmental area, and the brainstem.[23] Endocannabinoids are not stored in vesicles, but are produced and released 'on demand' only when and where they are needed. Consequently, they ideally inform about the incessant changes in energy availability. Not surprisingly, levels of endocannabinoids change in relation to the organism's energy status, increasing with fasting and decreasing during refeeding in both the rat hypothalamus and limbic forebrain. Besides, when AEA and 2-AG are directly injected within the hypothalamus or the NAc, they increase food intake through a CB_1 receptor-dependent mechanism.[24,25] Direct administration of AEA into the NAc shell increases the liking and the intake of a sucrose solution in rats in a CB_1 receptor-dependent manner.[26] Cannabinoids like Δ^9-tetrahydrocannabinol facilitate hedonic taste responses by increasing the dopamine release in the NAc shell after sucrose exposure.[27] However, administration of the CB_1 receptor antagonist rimonabant prevents the increase in dopamine release in the NAc shell that is classically associated with the consumption of a novel palatable food.[28] This evidence therefore suggests that the intake of palatable food increases endocannabinoids levels in the NAc shell and that such increase induces dopamine release in this brain area. As for the exact circuits determining this set of responses, it is likely that activation of ventral tegmental area dopaminergic neurons is achieved through an endocannabinoid-dependent activation of CB_1 receptors on glutamatergic terminals that, by inhibiting GABAergic neurons projecting from the NAc to the ventral tegmental area, disinhibit dopaminergic neurons in the ventral tegmental area.[29]

Taste-related neuronal signals coming from the oral cavity are processed in the parabrachial nucleus and in the nucleus of the solitary tract in the hindbrain, where they are integrated with information coming from the gastrointestinal tract and modulate meal size and intermeal intervals.[23] Endocannabinoids act through CB_1 receptors located in the parabrachial nucleus to specifically increase intake of palatable food.[30] Thus, as it happens in reward-related circuits, CB_1 receptor signaling in the parabrachial nucleus facilitates the intake of food with hedonically positive sensory properties.[30] Besides, food intake can also be favored by the action of CB_1 receptor-dependent signaling on olfactory circuits. In fact, recent work by Soria-Gomez *et al.*[31] has shown that fasting induces an increase in endocannabinoid levels in the olfactory bulb, activating CB_1 receptors on olfactory cortex axon terminals and inhibiting granular cells in the olfactory bulb, increasing odor detection and food intake. Thus, although evidence is still missing, it is possible that deregulation of endocannabinoid-dependent taste-related and olfaction-related neuronal responses might have a role in obesity.

CB_1 receptor mRNA is also found in several hypothalamic neuronal populations participating in the control of food intake and body weight,[19] and the role of hypothalamic CB_1 receptor signaling has been often investigated in association with the

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action of hormones known to play a role in energy balance, such as the anorexigenic hormone leptin and the orexigenic hormones glucocorticoids and ghrelin.

Leptin negatively regulates hypothalamic endocannabinoids levels, whereas genetic obese models with defective leptin production or signaling have increased hypothalamic endocannabinoid levels.[32] Leptin prevents endocannabinoid synthesis by reducing intracellular calcium levels, a mechanism that explains leptin's ability in inhibiting CB_1-dependent activation of orexigenic melanin-concentrating hormone-expressing neurons in the lateral hypothalamus.[33] However, leptin requires hypothalamic CB_1 receptor signaling to exert its anorexigenic effect, as partial deletion of hypothalamic *CB1* leads to the inability of the hormone to decrease food intake in mice.[34] The ability of leptin to modulate food intake and metabolism actually depends on CB_1 receptor signaling in specific neuronal populations and on the type of diet ingested. In fact, although deletion of CB_1 receptors in steroidogenic factor-1 (SF1)-expressing neurons of the ventromedial hypothalamus increases sensitivity to the anorexigenic and metabolic effects of leptin during consumption of regular chow, lack of CB_1 in SF1-expressing neurons causes molecular leptin resistance during consumption of a high-fat diet.[35] In addition, recent studies have shown that presynaptic inputs expressing CB_1 receptors change from being excitatory to inhibitory in hypothalamic orexin neurons when mice are fed a high-fat diet.[36] This neuronal rewiring is in part due to impairment in leptin signaling and causes an increased activation of orexin neurons that in turn might increase food intake and body weight.[36] Hence, this set of recent studies has further detailed the complex relationship between leptin and endocannabinoids within the hypothalamus.

Besides, an interaction also exists between leptin and glucocorticoids in the regulation of endocannabinoid synthesis in the paraventricular nucleus (PVN). Glucocorticoids act via a membrane receptor to quickly cause endocannabinoid-mediated suppression of synaptic excitation in PVN neurosecretory neurons.[37] This mechanism is used by glucocorticoids to rapidly inhibit hypothalamic hormone secretion.[37] Leptin blocks glucocorticoid-induced endocannabinoid biosynthesis and suppression of excitation in PVN neurons.[38] Interestingly, hypothalamic increase in endocannabinoid signaling not only interferes with leptin's actions, but can also lead to peripheral insulin resistance. Indeed, hyperinsulinemic, euglycemic clamp studies have demonstrated that central activation of CB_1 receptors is sufficient to impair glucose homeostasis by hampering insulin action in the liver and the adipose tissue.[39]

Finally, the hormone ghrelin and the ECS share several commonalities in the regulation of energy balance. Endocannabinoids mediate the orexigenic effect of ghrelin, when this hormone is administered in the PVN.[40] Ghrelin actually requires functional CB_1 receptor signaling that in turn may recruit the AMP-activated protein kinase, an intracellular fuel gauge whose activity is necessary for the action of ghrelin within the hypothalamus.[41,42] Of note, AEA favors ghrelin synthesis and secretion from the rat stomach.[43] However, in normal-weight humans, the consumption of food for pleasure has been associated with increased ghrelin and 2-AG plasma levels,[44] implying a close link between the ECS and the ghrelin system in the regulation of reward-related responses.

As for the exact role of CB_1 receptor signaling in the regulation of food intake and energy balance, this varies upon the specific circuits on which the CB_1 exerts its function. Recent studies have demonstrated that CB_1 receptor activation has opposite effects on food intake depending on whether CB_1 receptors are localized on presynaptic terminals of excitatory or inhibitory neurons.[45] Thus, although the well-known orexigenic effect of the endocannabinoids seems to depend upon actions at CB_1 receptors located at the terminals of cortical glutamatergic neurons, ventral striatal CB_1 receptors exert a hypophagic action through the inhibition of GABAergic transmission.[45] However, in order to explain the well-known orexigenic effects of CB_1 receptor agonists and the anorexigenic effects of CB_1 receptor antagonists, one must deduce that CB_1 receptor-dependent inhibition of glutamatergic signaling predominates over the action on GABAergic signaling.

Investigations carried out in our laboratory have also demonstrated that virally mediated knockdown of CB_1 mRNA expression within the adult mouse hypothalamus causes a lean phenotype by increasing energy expenditure, while not altering basal food intake.[34] In other studies, we have shown that basal food intake is not modified in chow-fed mice lacking CB_1 in Single-minded homolog 1 (Sim1)-expressing neurons,[46] which constitute the majority of neurons of the PVN, or in mice lacking CB_1 receptors in SF1-expressing neurons of the ventromedial hypothalamus.[35] Interestingly, chow-fed SF1-CB_1-knockout (KO) mice are hypophagic when reexposed to food after a prolonged fast,[47] but are hyperphagic when fed a high-fat diet.[35] Hence, this latest evidence suggests that CB_1 receptor signaling in SF1-expressing neurons has opposite effects on feeding behavior that depends upon interaction with other signals (that is, hormones and nutrients) relevant to the regulation of fasting-induced food intake, and upon the type of diet consumed. Indeed, the diet can importantly affect the ECS and its function within the CNS, as endocannabinoids are products of phospholipid-derived arachidonic acid, whose levels can be modified in response to n-3 and n-6 PUFAs present in the diet.[48] For instance, lifelong n-3 PUFAs dietary insufficiency abolishes endocannabinoid-dependent long-term synaptic depression, a form of synaptic plasticity, in the NAc and prelimbic prefrontal cortex.[49] This is due to the inability of presynaptic CB_1 receptors to respond to endocannabinoids because of the uncoupling of their effector Gi/o proteins.[49]

Altogether, the data reviewed above clearly demonstrate that the ECS regulates feeding behavior by acting upon neuronal circuits located in reward-related structures, the hindbrain and the hypothalamus, and that its activation favors the intake of calories, particularly from palatable food. Recent evidence has further clarified that the net action of endocannabinoids on food intake depends on the neuronal type (that is, glutamatergic vs GABAergic) and that the diet might affect CNS endocannabinoid signaling.

However, the role of ECS in CNS regulation of energy balance might not be limited to the modulation of the activity of CB_1 receptors located at the level of the neuronal membrane. CB_1 receptors are also present in mouse brain mitochondrial membranes, where they regulate neuronal energy metabolism and endocannabinoid-dependent neurotransmitter release.[50,51] In addition, CB_1 receptors are present in astrocytes,[52,53] cells that are being recognized to have important functions in the regulation of energy balance.[54] Intriguingly, astroglial CB_1 receptors directly interfere with leptin signaling and its ability to regulate glycogen storage, thereby representing a novel mechanism regulating brain energy storage.[53] Whether this recently discovered mechanism may affect whole body energy balance is, however, presently unknown.

THE ECS AND THE CENTRAL AND PERIPHERAL INTEGRATION OF ENERGY BALANCE

In order to integrate the information coming from the periphery and appropriately coordinate intake, storage and use of calories, the brain must continuously communicate with peripheral organs. Several recently published studies have underscored the ability of CNS endocannabinoid-dependent mechanisms to modulate peripheral processes such as energy expenditure, thermogenesis and lipolysis.[34,35,46,55–57] Mice with selective knockout of CB_1 receptors in the forebrain and sympathetic neurons are resistant to diet-induced obesity because they display increased lipid oxidation and thermogenesis as a consequence of enhanced

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sympathetic nervous system (SNS) activity associated with a decrease in energy absorption.[56] Virally mediated knockdown of CB_1 receptor mRNA in the adult mouse hypothalamus also causes a decrease in body weight gain because of an increase in energy expenditure.[34] In addition, deletion of CB_1 receptors from Sim1-expressing neurons protects from diet-induced obesity by increasing the expression of thermogenic genes in the brown adipose tissue and by inducing energy expenditure.[46] These modifications seem to be due to increased SNS activity, as pharmacological blockade of β-adrenergic receptors or chemical sympathectomy respectively blunt energy expenditure increase and abolish the obesity-resistant phenotype of Sim1-CB_1-KO mice.[46] Similarly, deletion of CB_1 receptors from SF1-expressing neurons protect chow-fed mice from body weight gain by inducing brown adipose tissue thermogenic activity and lipolysis in white adipose tissue (WAT) through heightened SNS activity.[35] Accordingly, hypothalamic CB_1 signaling is involved in determining the thermogenic effects of MC4R (melanocortin-4 receptor) agonists.[58] Genetic models characterized by increased hydrolysis of 2-AG in the forebrain also show increased SNS-mediated brown adipose tissue thermogenesis and mitochondrial density and consequent resistance to diet-induced obesity.[59] Thus, the set of recent studies reviewed above has clearly established a link between the ECS in the CNS, the SNS and the regulation of energy balance. Of note, the rapid (within 1 h) hypophagic effect caused by the peripheral administration of rimonabant not only requires peripheral sensory nerve terminals but also depends upon SNS activity.[47,60] In particular, we recently demonstrated that rimonabant-induced hypophagia is fully abolished by peripheral blockade of β-adrenergic transmission.[47] The latter also inhibited central effects of CB_1 receptor blockade, such as fear responses and anxiety-like behaviors, suggesting that, independently of where they originate from, behavioral effects of CB_1 receptor antagonism are expressed via activation of peripheral sympathetic activity.

THE ECS AND THE GASTROINTESTINAL TRACT

When food is introduced into the mouth, it is sensed by the taste buds located on the papillae of the tongue. CB_1 receptors are colocalized with type 1 sweet taste receptor 3 on the mouse tongue, and CB_1 receptor-dependent endocannabinoid signaling specifically enhances neural responses to sweet taste, as peripheral administration of endocannabinoids increases the neural activity elicited in the chorda tympani by sweeteners, but not by bitter, umami, salty or sour compounds.[61] This effect can also be observed *in vitro*, by applying endocannabinoids directly to taste cells, suggesting that local endocannabinoid signaling in the oral cavity modulates sensitivity to sweet taste.[61] Accordingly, AEA, 2-AG and related *N*-acylethanolamines produced together with endocannabinoids such as oleoylethanolamide and palmitoylethanolamide are quantifiable in human saliva, with their levels being higher in obese patients than normal-weight subjects.[62] Although further studies are needed, it is possible that salivary endocannabinoids might play a role in the modulation of orosensory information. In particular, the composition of the meal and the specific presence of fat might affect salivary endocannabinoid and *N*-acylethanolamines pools that in turn might modulate taste signaling.

Of note, recent studies have demonstrated a link between the ECS and cephalic phase responses elicited in anticipation of a meal to enhance its digestion and metabolism. Cephalic phase responses can be studied in rodents using for instance the sham-feeding model in which the effects of the orosensory properties of the food can be separated from its postingestive qualities. Using this experimental model, it has been shown that gut-derived endocannabinoids regulate the intake of fat based on its orosensory properties.[63,64] Sham-feeding rats with a high-fat liquid meal increases AEA and 2-AG levels specifically in the jejunal part of the small intestine.[63,64] The intestinal increase in endocannabinoids in turn induces food consumption, as local pharmacological blockade of CB_1 receptors in the small intestine just before sham-feeding inhibited food intake.[63] Transection of the vagus nerve prevents the sham-feeding effect on gastrointestinal endocannabinoids, implying that signals that originate in the oral cavity are transmitted to the brainstem, and then through the vagus to the intestine, where they induce the production of endocannabinoids.[3,63] Certain types of fatty acids may actually be responsible for the cephalic phase of gut endocannabinoid production, as sham-feeding emulsions containing oleic acid or linoleic acid caused a nearly twofold accumulation of jejunal endocannabinoids.[64] Besides, mobilization of endocannabinoids in the gut may be essential for fat preference, as suggested by the observation that rats in a two-bottle-choice sham-feeding test displayed strong preference for emulsions containing linoleic acid that was blocked by the administration of a peripherally restricted CB_1 receptor antagonist.[64] Intriguingly, it has been proposed that a greater intake of food rich in linoleic acid rather than saturated fats might be one of the underlying causes of obesity, as the presence of linoleic acid in western diet has increased over this past century and is positively associated with the increase in obesity in rates.[65]

Thus, the evidence reviewed above has clearly established that gut-produced endocannabinoids importantly affect fat intake and preference. Recent studies have also revealed that gut microbiota, which is known to influence energy balance and metabolism, controls the gastrointestinal ECS tone.[66,67] The latter can increase gut permeability that, by augmenting lipopolysaccharide levels, further exacerbates the ECS tone in both the gastrointestinal tract and the WAT, favoring body weight gain.[67] However, additional investigations are needed in order to better clarify the relationship between gut microbiota and the ECS, particularly as later, apparently contradictory, findings have demonstrated that beneficial gut microbiota, which protects from fat mass gain and insulin resistance, actually increases 2-AG content in the ileum.[68]

THE ECS IN OTHER PERIPHERAL ORGANS AND CIRCULATING ENDOCANNABINOIDS

After its initial discovery, the CB_1 receptor was known as the brain cannabinoid receptor. This nomenclature had to change in 2003 when groundbreaking work by Cota *et al.*[19] and Bensaid *et al.*[69] demonstrated the functional presence of the CB_1 receptor on white adipocytes.

Since then, the role of peripheral CB_1 in adipocytes as well as in hepatocytes, pancreas and skeletal muscle has been deeply investigated and better characterized, opening new perspectives for the treatment of metabolic disorders.

CB_1 receptor activation in white adipocytes increases the expression of genes associated with adipocyte differentiation, such as peroxisome proliferator-activated receptor-γ (PPARγ), and impairs mitochondrial biogenesis.[70,71] Conversely, pharmacological blockade or genetic deletion of CB_1 receptors stimulates mitochondrial biogenesis through the increased expression of the endothelial nitric oxide synthase,[72] and induces the transdifferentiation of white adipocytes into a thermogenic brown fat phenotype characterized by increased UCP-1 (uncoupling protein-1) and PGC-1α (peroxisome proliferator-activated receptor-γ coactivator 1α) expression and enhanced AMP-activated protein kinase activity.[73] Similarly, CB_1 receptor blockade activates brown adipocytes, resulting in enhanced uncoupled respiration.[74]

White adipocyte CB_1 receptor activation causes increased fatty acid synthesis and triglycerides accumulation, whereas the opposite is observed when pharmacologically blocking or genetically deleting this receptor.[19,69,75] Moreover, endocannabinoid production in white adipocytes is negatively regulated by

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insulin and leptin.[75,76] Consequently, conditions characterized by leptin and insulin resistance, such as diet-induced obesity, might favor ECS overactivity in the WAT that in turn might further support fat accumulation and body weight gain. However, controversy exists on whether the above described changes in adipocyte function, which are due to the modulation of adipocyte CB_1 receptor activity *in vitro*, are relevant *in vivo*, particularly if one considers studies that have demonstrated that the ECS regulates WAT lipogenesis/lipolysis through the SNS and not at tissue level.[77] Nevertheless, findings obtained using adipocyte-specific CB_1-KO mice do suggest that adipocyte CB_1 receptors favor WAT expansion and the development of obesity *in vivo*.[78]

Similar to white adipocytes, activation of CB_1 receptors in hepatocytes causes lipid accumulation and leads to liver steatosis by inducing the expression of lipogenic enzymes, such as ACC1 (acetyl coenzyme-A carboxylase-1) and fatty acid synthase, and by increasing *de novo* fatty acid synthesis.[79] Mice with specific deletion of CB_1 receptors in hepatocytes, although still gaining body weight when consuming a high-fat diet, do not develop liver steatosis, hyperglycemia, dyslipidemia and insulin resistance.[80] Similarly, the beneficial effects of a peripherally restricted CB_1 receptor antagonist on liver steatosis and insulin resistance depend upon the action of the compound on hepatocyte CB_1 receptors.[81,82] Thus, these recent findings imply that endocannabinoid-dependent CB_1 receptor signaling in hepatocytes may have a particularly relevant role in the regulation of lipid metabolism and insulin sensitivity.

At the molecular level, activation of hepatic CB_1 receptors favors insulin resistance through the upregulation of the inhibitory phosphorylation of the insulin receptor substrate and of the inhibitory dephosphorylation of the insulin-activated protein kinase B with consequent recruitment of an endoplasmic reticulum stress-dependent pathway.[83] A number of studies have also implicated both CB_1 and CB_2 receptors in the regulation of fibrogenic responses in the liver and the reader should refer to recent reviews that have specifically addressed the role of the ECS in liver physiopathology.[84]

Both CB_1 and CB_2 receptors are present in rodent and human pancreatic islets and show species-dependent degree of expression, with CB_1 receptor signaling regulating both insulin signaling and insulin release.[85] A series of recent investigations has in particular detailed the role of CB_1 in the β-cell. Activation of CB_1 receptors in β-cells has been shown to recruit focal adhesion kinases that lead to exocytosis of secretory insulin vesicles through cytoskeletal reorganization.[86] Conversely, pharmacological CB_1 receptor blockade inhibits insulin secretion *in vitro* only when elevated above normal, as it can be found in diet-induced obesity, indicating that under this condition there is a higher endocannabinoid tone in the endocrine pancreas, similar to what is described in other organs. CB_1 receptor blockade also ameliorates β-cell function in obesity by increasing β-cell proliferation and mass.[87] Conversely, CB_1 receptor activation induces apoptotic activity and β-cell death.[88] Actually, it has been recently demonstrated that macrophage infiltration of pancreatic islets and consequent inflammation, which plays a pathogenic role in diabetes, is under the control of CB_1 receptor activity and leads to β-cell loss in type 2 diabetes.[89] Accordingly, peripheral CB_1 receptor blockade, *in vivo* depletion of macrophages or macrophage-specific knockdown of the CB_1 receptor restores normoglycemia and glucose-induced insulin secretion.[89] Therefore, these latest findings imply that pharmacological interventions aimed at inhibiting peripheral CB_1 receptors might represent new attractive avenues for the treatment of diabetes, independently of their possible beneficial effects on body weight, lipid metabolism and insulin resistance.

Endocannabinoids and the different ECS components are also present in the skeletal muscle. Muscle endocannabinoids and muscle CB_1 receptor expression are altered by high-fat diet consumption and in animal models of obesity,; although changes (increase or decrease) might depend on the specific muscle or genetic model studied (reviewed in Silvestri and Di Marzo[4]). Some data imply that overactivity of the ECS in the skeletal muscle might drive defective oxidative metabolism, as activation of the ECS in muscle inhibits oxidative pathways and mitochondrial biogenesis.[71] Moreover, CB_1 receptor activation in isolated soleus muscle from either lean or obese animals hampers both basal and insulin-stimulated glucose transport, whereas pharmacological blockade of CB_1 improves glucose transport.[4] In particular, activation of muscle CB_1 receptor negatively affects the responsiveness of the tissue to insulin through the phosphatidylinositol 3-kinase/protein kinase B and the Raf-MEK1/2-ERK1/2 intracellular pathways, among other molecular cascades (reviewed in Silvestri and Di Marzo[4]), suggesting that CB_1-driven changes in muscle might favor insulin resistance.

Finally, endocannabinoids can be detected in the circulation, and measurement of endocannabinoids in the plasma or serum has been a favorite approach for the study of the ECS in humans (see also Table 1). Plasma endocannabinoid levels correlate positively with markers of obesity and metabolic disorder in humans, such as the body mass index, the waist circumference, visceral fat mass and insulin resistance.[90–93] It has actually been recently suggested that circulating endocannabinoids might work as biomarkers of WAT distribution and insulin resistance.[4] Consequently, they might be used not only as markers of specific phenotypes, but also to predict responsiveness to treatment. However, several issues for the clinical use of circulating endocannabinoids still remain unresolved, including the requirement for standardized methods dealing with the extraction and the measurement of endocannabinoids and the establishment of reference levels for plasma/serum endocannabinoids in humans that might be affected by age and gender, among other factors.[94] Other points that currently require further investigation include the possible participation of circulating endocannabinoids in signaling events that might affect feeding and metabolism, and the actual origin(s) of circulating endocannabinoids. Circulating levels of endocannabinoids change in healthy and obese humans in relation to food intake.[93,95] In particular, we have demonstrated that both normal-weight and obese subjects have a significant preprandial AEA peak, a finding implying that AEA might work as a physiological meal initiator.[93] Differently from AEA, no meal-related changes were found for 2-AG, suggesting that 2-AG might not work as a hunger signal in humans.[93] As to from where the observed changes in AEA and 2-AG plasma levels originate, we have proposed as a probable candidate the gastrointestinal tract,[93] as it produces endocannabinoids in relation to food intake.[63]

THE ECS AS A TARGET FOR THE TREATMENT OF OBESITY AND METABOLIC DISORDERS

Evidence reviewed above pinpoints the CB_1 receptor as the best characterized potential pharmacological target through which the ECS can be modulated in obesity and metabolic disorders. This has been due in great part to the fact that rimonabant, the first systemically penetrant CB_1 receptor inverse agonist, was developed soon after the discovery of the CB_1 receptor.[96] Chronic administration of rimonabant in obese rodents and humans reliably decreases body weight and fat mass, improves glucose homeostasis and ameliorates insulin sensitivity and associated cardiometabolic risks.[97] However, the neuropsychiatric side effects, which were more common than what were initially estimated from the clinical trials, and the overall benefits being lower than the risks, led to the withdrawal of rimonabant from the market and to the dismissal of similar CB_1 receptor antagonists. Yet, as the ECS is strategically positioned to regulate every step affecting the intake, storage and use of calories, research over the

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Table 1. Quantification of endocannabinoids and related-compounds in human subjects suffering from obesity and/or cardiometabolic disorders

Condition	*Subjects*	*Measurements*	*Results*	*References*
Obesity	20 Normal weight; 20 obese	Plasma AEA and 2-AG levels	↗AEA and 2-AG in obese vs normal weight	90
	10 Normal weight; 10 obese with subcutaneous obesity; 10 obese with visceral obesity	Plasma AEA and 2-AG levels	↗AEA in obese vs normal weight ↗2-AG in visceral vs subcutaneous obesity	91
	10 Normal weight; 10 hyperinsulinemic obese	Plasma AEA and 2-AG levels before and after OGTT	↗AEA and 2-AG levels after OGTT only in normal weight	109
	48 Normal weight; 96 obese	Plasma AEA levels Polymorphisms of FAAH	↗ Plasma AEA (trend) in obesity	110
	27 Individuals of different race	Plasma and CSF AEA, 2-AG, OEA and PEA levels	Positive correlation between plasma AEA levels and adiposity Negative correlation between plasma AEA levels and adiposity	111
	12 Normal weight; 12 hyperinsulinemic obese	Plasma AEA and 2-AG levels before and after a meal	Postprandial reduction of plasma AEA in normal weight vs hyperinsulinemic obese	93
	12 Normal weight; 12 obese	Salivary AEA, 2-AG, OEA and PEA levels	↗AEA in obese vs normal weight	62
Eating disorders	15 Healthy control; 11 patients with AN; 8 patients with BN; 11 patients with BED	Plasma AEA and 2-AG levels	↗AEA in AN and BED vs healthy	112
Type 2 diabetes	8 Healthy control; 10 diabetic patients	Plasma AEA and 2-AG levels	↗AEA and 2-AG in diabetic vs healthy	75
	12 Healthy control; 7 diabetic patients	Plasma AEA and 2-AG levels	No difference in AEA and 2-AG between subjects	109
	19 Normal weight/overweight males including type 2 diabetic	Plasma AEA and 2-AG levels before and after insulin infusion	Insulin-dependent reduction of AEA and 2-AG levels inversely related to anthropometric and metabolic predictors of insulin resistance	109
Sleep apnea syndrome	57 Healthy controls; 20 patients with sleep apnea	Plasma AEA, 2-AG and OEA levels	↗Plasma OEA in patients with sleep apnea	113
	21 Obese with no sleep apnea; 55 obese with sleep apnea	Plasma AEA, 1-/2-AG, AA and OEA levels	↗Plasma AEA 1/2-AG and OEA levels in obese patients with sleep apnea	114
Coronary circulatory dysfunction	21 Normal-weight controls, 26 overweight subjects; 30 obese subjects	Plasma AEA and 2-AG levels	↗Plasma AEA and 2-AG significantly associated with impairment of coronary circulatory function in obese subjects	115

Abbreviations: AA, arachidonic acid; AEA, anandamide; 2-AG, 2 arachidonoylglycerol; 1-/2-AG, the sum of 1-arachidonoylglycerol and 2-arachidonoylglycerol; AN, anorexia nervosa; BED, binge eating disorders; BN, bulimia nervosa; CSF, cerebrospinal fluid; FAAH, fatty acide amine hydrolase; OEA, oleoylethanolamide; OGTT, oral glucose tolerance test; PEA, palmitoylethanolamide. The ↗ means increased.

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Table 2. Therapeutic strategies targeting the ECS that are currently under investigation for the treatment of obesity and metabolic disease

Intervention	*Target population*	*Cardiometabolic effects*	*Effects on ECS tone*	*Central side effects*	*References*
Diet enriched in PUFA	HFD mice	Not assessed	Decreased tissular ECs	Not assessed	116
	Overweight and obese humans	No changes	Decreased plasma 2-AG	Not assessed	117
	Hypercholesterolemic humans	Improvement of lipid metabolism	Decreased plasma AEA	Not assessed	105
CB_1 neutral antagonists	Normal-weight rats	Reduction of weight; reduction of food intake	Not assessed	No	118
Peripherally restricted CB_1 antagonists	Obese rodents	reduction of weight; Improvement of glucose metabolism; improvement of lipid metabolism	Not assessed	No	81,82,119
CB_1 allosteric inhibitors					
PSNCBAM-1	Chow-fed rats	Reduction of body weight and food intake	Not assessed	Not assessed	120
Hemopressin	Chow-fed rats and mice; *ob/ob* mice	Reduction of body weight and food intake	Not assessed	No	98
Pregnenolone	Diet-induced obese mice	Reduction in body weight and fat mass	Not assessed	No	99
Combinatorial treatment	Lean and obese mice	Reduction of food intake; reduction of body weight; increased thermogenesis	Not assessed	Improved	106,107

Abbreviations: AEA, anandamide; 2-AG, 2-arachidonoylglycerol; CB_1, cannabinoid type 1; EC, endocannabinoid; ECS, endocannabinoid system; HFD, high-fat diet; PUFA, polyunsaturated fatty acid.

past 5 years has intensely and successfully pursued the development of novel approaches to modulate ECS activity. These strategies, summarized in Table 2, are based on very different and equally attractive approaches either targeting CB_1 with peripherally restricted antagonists or allosteric inhibitors or neutral antagonists or directly targeting the endogenous ligands through the diet or inhibitors of endocannabinoid synthesis. In particular, Tam *et al.*[81,82] were the first to characterize the metabolic effects of AM6545, a peripherally restricted CB_1 receptor neutral antagonist, that does not alter behavioral responses mediated by CNS CB_1 receptors, while reversing leptin resistance and inducing weight-independent improvements in glucose homeostasis, liver steatosis and plasma lipid profiles in genetic or diet-induced obese mice. However, very recent findings have suggested that endogenous compounds, such as hemopressin or the neurosteroid pregnenolone, that are able to act as allosteric inhibitors of CB_1 might avoid causing side effects because of modulating CB_1 receptor activity in a signaling-specific way and/or by engaging distinct neuronal substrates within the CNS.[98–102] Thus, pharmacological compounds mimicking the actions of endogenous allosteric inhibitors might represent a novel, efficacious way to modulate ECS activity in pathology. Although targeting CB_1 receptors for the treatment of obesity and metabolic disorders remains the option with most descriptive and mechanistic information available, alternative possibilities exist. For instance, recent studies suggest a metabolic role for CB_2 receptors that might be an interesting target for the treatment of at least some metabolic disorders, particularly considering the function of this receptor in the regulation of immune and inflammatory responses.[4,84] Additional therapeutic potential targets are also non-cannabinoid receptors that can be activated by endocannabinoids, such as the TRPV1 (transient receptor potential vanilloid 1) and the PPARs. However, further work is required in order to better understand the role of these receptors in the context of endocannabinoid-dependent regulation of energy balance and metabolism.

As obesity is characterized by upregulation of the endocannabinoid tone, which can be in part attributed to increased endocannabinoid synthesis, other therapeutic possibilities are given by compounds that can interfere with endocannabinoid synthesis or that increase endocannabinoid degradation. For instance, diacylglicerol lipase inhibitors, which decrease 2-AG synthesis, inhibit food intake and body weight in mice.[103] Another therapeutic possibility is the decrease in the availability of endocannabinoid precursors. This could be attained by increasing the levels of dietary n-3 PUFAs. In fact, it has been demonstrated that the increased intake of dietary n-3 PUFAs in obese rats reduces endocannabinoid levels in adipose tissue, liver and heart, limiting ectopic fat accumulation and inflammatory responses.[104] Similarly, hypercholesterolemic patients consuming sheep cheese enriched in n-3 PUFAs for 3 weeks have decreased plasma AEA levels and improved lipid profile.[105] Thus, increasing the dietary consumption of n-3 PUFAs might regulate endocannabinoid levels so to help in preventing or treating metabolic disorders. Finally, considering the complexity and redundancy of the mechanisms regulating energy balance, the ECS could be targeted in association with other biological systems.[106,107] This strategy is an extremely attractive approach for the treatment of obesity that, by preferring the combination of low doses of compounds targeting different systems or different components of the same system, helps limiting the appearance of unwanted side effects.

CONCLUDING REMARKS: THE CHALLENGES AHEAD

Research over the past 5 years has significantly expanded our knowledge about the ECS and its role in the regulation of energy balance. In particular, new evidence has shown an involvement of

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the ECS in the modulation of taste and olfaction and the role of this system at the level of the gastrointestinal tract in the regulation of fat intake and preference. Other studies have proven the function of the CNS ECS in the regulation of peripheral metabolism, and further knowledge has been provided on the actions of the peripheral ECS and its impact particularly on glucose metabolism and insulin sensitivity. The main conclusion is that the ECS generally acts to preserve and stock energy in the body. Thus, although activation of this system is beneficial in conditions in which the availability of food is limited or cannot be predicted, it favors the development of obesity and metabolic syndrome when palatable, calorically rich food becomes easily available.

Here we have mainly focused on the functions of AEA, 2-AG and CB_1 receptors, as a great part of the information concerning the role of the ECS in energy balance is related to these endocannabinoids and to this specific cannabinoid receptor. However, we need to mention that endocannabinoids can affect metabolic responses by acting on receptors (that is, PPARs or TRPV1) other than cannabinoid receptors. In addition, endocannabinoids are synthetized together with other bioactive lipids that, while sharing structural similarities with the endocannabinoids, do not bind to cannabinoid receptors. Among those, oleoylethanolamide, a lipid related to AEA, decreases appetite and favors weight loss and lipolysis by acting through PPARα, contrasting the metabolic effects of endocannabinoid-dependent CB_1 receptor activation.[108] Thus, the information generated so far on the ability of endocannabinoids to bind to receptors other than cannabinoid receptors, the redundancy of their metabolic pathways, the presence of endocannabinoid-related compounds that oppose the actions of endocannabinoids and the identification of endogenous modulators of CB_1 receptor activity represent a complicated scenario that asks for substantial additional investigation in order to fully grasp the impact of the ECS in energy balance and metabolism. Apart from these challenges, available evidence suggests that the ECS remains an attractive target for therapy. Thus, there is strong hope that new therapeutic approaches targeting the ECS might successfully help tackling obesity and metabolic disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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