

Review Article

Endocannabinoids and energy homeostasis: An update

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Abstract

The endocannabinoid system (ECS) is a widespread intercellular signaling system that plays a critical role in energy homeostasis, meant as the precise matching of caloric intake with energy expenditure which normally keeps body weight stable over time. Complex interactions between environmental and neuro-hormonal systems directly contribute to the balance of energy homeostasis. This review highlights established and more recent data on the brain circuits in which the ECS plays an important regulatory role, with focus on the hypothalamus, a region where numerous interacting systems regulating feeding, satiety, stress, and other motivational states coexist. Although

not meant as an exhaustive review of the field, this article will discuss how endocannabinoid tone, in addition to reinforcing reward circuitries and modulating food intake and the salience of food, controls lipid and glucose metabolism in several peripheral organs, particularly the liver and adipose tissue. Direct actions in the skeletal muscle and pancreas are also emerging and are briefly discussed. This review provides new perspectives into endocannabinoid control of the neurochemical causes and consequences of energy homeostasis imbalance, a knowledge that might lead to new potential treatments for obesity and related morbidities. © 2014 BioFactors, 40(4):389–397, 2014

Keywords: hypothalamus; leptin; retrograde neurotransmission; metabolic disfunctions; anti-obesity drugs

1. Introduction

Energy homeostasis ultimately refers to the state in which energy intake equals energy expenditure. In case energy intake or expenditure exceeds the other, obese or anorectic phenotypes, respectively, would be the consequence. In order to maintain a well balanced energy status the brain needs pathways that are able to detect changes in the body energy budget and effect a counteraction. In case of a drop in energy intake accompanied by constant or increased energy expenditure, brain pathways would stimulate food intake and promote

weight gain through orexigenic pathways. When energy balance is tipped onto the other side of the spectrum, anorexigenic pathways would cause the depletion of body fat and reduce energy intake [1]. It is becoming clear that complex interactions between the environment and neurohormonal systems affect directly the balance between the orexigenic and anorexigenic pathways that occur in the hypothalamus. This brain region operates as a highly sophisticated neuroendocrine transducer, sensing peripheral endocrine signals (*i.e.*, insulin and leptin) and transforming them into changes in intracerebral excitatory or inhibitory neurotransmitter signaling, which in turn deliver a homeostatic response back to the periphery through highly elaborated feed-back control loops. The endocannabinoid system (ECS) plays a critical role in energy homeostasis and 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 [2,3].

The ECS is composed of the two G_{V/o} protein-coupled “cannabinoid” CB1 and CB2 receptors, their lipid ligands (*i.e.*, the endocannabinoids, ECs), and the enzymatic machinery for

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EC synthesis and degradation. The most studied ECs, *N*-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), are members of the fatty acid amide and monoacylglycerol families of neutral lipids, respectively, and are produced from cell membrane phospholipids following cell stimulation, before being released to target cannabinoid receptors (reviewed in ref. 4). Among the chemical mediators involved in energy homeostasis, the ECs are emerging as master regulators of the fast (i.e., non-genomic) and stress-related fine-tuning of energy intake and processing because of their intrinsically rapid and adaptive mechanisms of synthesis and release. The ECS works as a local intercellular modulatory mechanism based on EC “tone,” which is mostly the result of the regulation of EC levels as determined by different, often concurring and hormone-regulated, enzymatic biosynthetic and catabolic cascades [5,6]. When a given stimulus (*e.g.*, membrane depolarization) reaches a cell equipped with the enzymes to synthesize ECs, these compounds are generated and then released into the extracellular space where they target, in an autocrine or paracrine manner, cannabinoid receptors. Activation of these receptors, generally, but not necessarily, coupled to inhibitory G proteins, leads to modulation of intracellular processes that eventually change the way cells respond to other stimuli. Examples of these modulations include decreased adenylyl cyclase activity, leading to lower cAMP production, and modulation of ion channel activity, such as activation of G-protein-coupled inwardly-rectifying potassium channels and inhibition of L-, N- and P/Q-type voltage-gated calcium channels. Also, different MAPK pathways are activated, such as ERK1/2, p38MAPK, and JNK [6]. The cannabinoid CB1 and CB2 receptors are differentially expressed in tissues and display different sensitivity to the ECs since 2-AG exhibits higher CB1 and CB2 efficacy than anandamide, the latter behaving as a high affinity partial agonist at both receptor types. The ECS is widely expressed throughout the body, participating critically in the maintenance of homeostasis [3], being an ideal candidate for responding, in real-time, to the ever changing requirements to which animals are submitted by the environment, such as food shortage. This is made possible since EC release occurs immediately after biosynthesis, usually following alterations in intracellular Ca^{2+} concentrations. Although peripheral mechanisms participate in the control of energy homeostasis by the ECS [7], the brain has emerged as the major organ involved in EC regulation of food intake.

2. Endocannabinoids and the Central Regulation of Energy Homeostasis: “Old” and “New” Concepts

In the brain, CB1 receptors are found predominantly at nerve terminals on which the ECs act in a retrograde manner to inhibit neurotransmitter release [5]. The hypothalamus is the key brain structure involved in energy homeostasis by inte-

grating the action of central orexigenic and anorexigenic neuropeptides with that of peripheral signals coming from the sympathetic system and peripheral organs deputed to sensing the status of energy stores [8–11]. Although hypothalamic CB1 expression is among the lowest in the brain, activation of the receptors shows high efficiency and leads to profound effects on the crosstalk between the hypothalamic nuclei and peripheral organs. Compelling evidence shows that ECs act as “gatekeepers” of the hypothalamic–pituitary–adrenal axis (HPA) constraining the activity of the latter under stressful conditions by lowering glucocorticoid levels. Indeed, in the hypothalamic dorsomedial (DMH) and periventricular (PVN) nuclei, stress and glucocorticoids can trigger EC synthesis and CB1 signaling to constrain HPA axis activity under acute conditions, whereas chronic or repeated stress leads to functional down-regulation of CB1 signaling (reviewed in ref. 12). Within the PVN, glucocorticoids, which have a known orexigenic action, rapidly inhibit magnocellular (which produce vasopressin and oxytocin) and parvocellular (which produce corticotropin releasing hormone [CRH] and thyrotropin-releasing hormone) neurons by suppressing excitatory glutamatergic synaptic inputs through retrograde release of ECs and presynaptic CB1 activation [13] (Fig. 1). In the arcuate nucleus (ARC), proopiomelanocortin (POMC) and Agouti-related peptide (AgRP) neurons, which regulate in opposite ways the activity of melanocortin receptors located in the PVN, are innervated by CB1-expressing GABAergic terminals. Remarkably, since ECs are constitutively produced by POMC cells, the anorexigenic action of the melanocortin system results tonically inhibited (reviewed in ref. 14) (Fig. 1). The neurons located in the lateral hypothalamus (LH) and expressing orexin (OX) or melanin-concentrating hormone (MCH) are mostly implicated in the regulation of energy balance [15]. In particular, the OX neurons might affect feeding behavior by modulating arousal and Huang et al. [16] found that CB1 agonists inhibit OX neurons while exciting MCH neurons. Finally, considerable evidence links the ECS with nuclear receptor steroidogenic factor 1 (SF-1)-expressing neurons of the ventromedial hypothalamus (VMH) in the regulation of energy homeostasis possibly by acting on thermogenesis by decreasing SF-1 neuron excitability [17] (Fig. 1).

EC levels become deregulated in the hypothalamus and nucleus accumbens (NAc) during obesity [18–20] being affected positively by orexigenic hormones, such as ghrelin and glucocorticoids and negatively by anorexigenic hormones, such as leptin and cholecystokinin. The hypothalamic concentrations of ECs, particularly 2-AG, increase during short food deprivation, return to normal after refeeding, and decrease after food consumption, without changing in brain areas not involved in feeding [21]. Activation of CB1 receptors increases feeding also in satiated rodents [22], whereas systemic pharmacological as well as genetic inhibition of CB1 results in hypophagia [18,23]. Interestingly, hypothalamic CB1 knockdown by 60% has no effects on basal food consumption, although the animals are less responsive to the hypophagic activity of the CB1 inverse

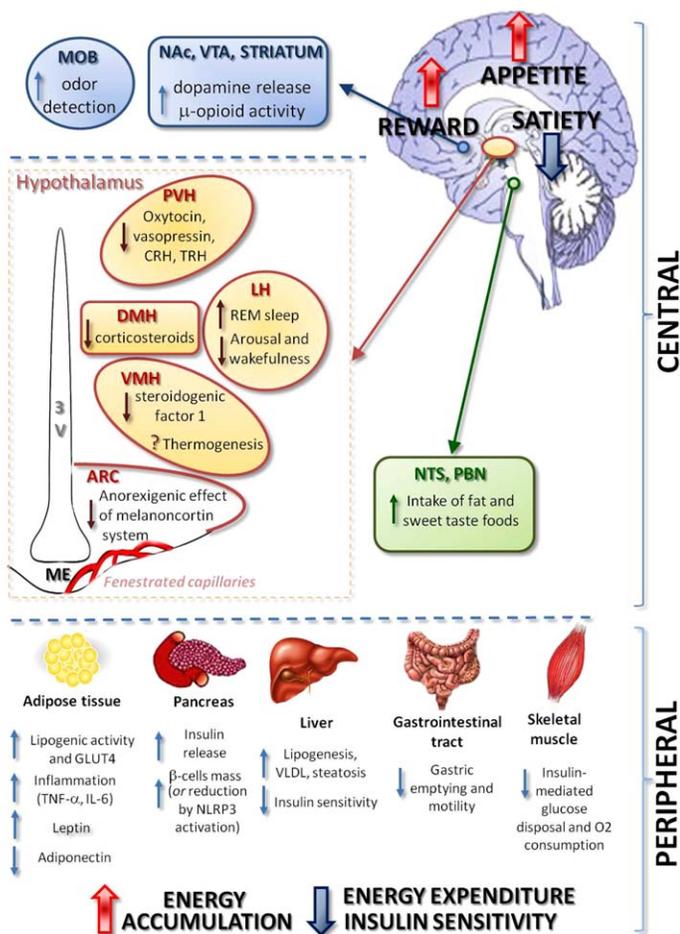


FIG 1

Effects of endocannabinoid overactivity on energy homeostasis. Functional relationships among the ECS, hypothalamic, cortico-limbic, and viscerosensory circuits and some peripheral signals participating in energy balance regulation. Please see the text for further details. Abbreviations: 3 V, third ventricle; ARC, arcuate nucleus; CRH, corticotropin-releasing hormone; DMH, dorsomedial hypothalamus; IL-6, interleukine 6; LH, lateral hypothalamus; ME, mediane eminence; MOB, main olfactory bulb; NAc, accumbens nucleus; NLRP3, nucleotide-binding oligomerization domain-like receptor 3; NTS, nucleus of the solitary tract; PBN, parabrachial nucleus; PVH, periventricular hypothalamus; TNF- α , tumor necrosis factor alpha; TRH, thyrotropin releasing hormone; VLDL, very low density lipoprotein; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

agonist rimonabant, and become insensitive to the anorexigenic actions of leptin [9], thus strengthening the physiological link between leptin and the ECS in the brain. This link was first shown in the pioneering study of Di Marzo et al., [18], who reported that intravenous leptin injection in rats decreases both AEA and 2-AG hypothalamic levels. After these initial studies on the positive action of ECS and CB1 on food intake, the regulation of food intake by the central ECS has proved to be more complex than initially thought because of emerging evidence of

a bimodal orexigenic as well as anorexigenic effect of CB1 receptors, depending on whether they are activated in glutamatergic or GABAergic terminals, respectively [24]. This complex function becomes even more intriguing when considering that the hypothalamus can be rapidly “rewired” in terms of what and how neurons are regulated by excitatory versus inhibitory inputs, in fasted versus ad-lib-fed or in lean versus obese animals, often as the result of changes in leptin or glucocorticoid signaling [25,26]. Indeed, we have recently reported that LH neurons producing OX-A, a peptide involved in reward- and appetite-related functions, during obesity undergo synaptic “rewiring” of their CB1-expressing afferences from predominantly excitatory to inhibitory, resulting in elevated retrograde disinhibition, rather than inhibition of activation by 2-AG and increased OX-A release in LH output areas. These alterations contribute to hyperphagia and hormonal dysfunctions typical of obesity, to increase of reward and reduction of sensitivity to nociceptive stimuli [27,28]. This “rewiring” is strictly leptin-sensitive, being: 1) observed both in obese leptin-deficient (*Lep^{ob/ob}*) mice and in mice made obese, and leptin-resistant in the ARC, after a prolonged high fat diet, and 2) reversed after leptin administration only in the former mice. Furthermore, orexinergic neurons synthesize and release 2-AG through diacylglycerol lipase- α (DAGL α), the expression of which is significantly upregulated in these obese mice.

As with the anorexigenic actions of leptin, also the orexigenic action of ghrelin in rodents is dependent on the presence of CB1 receptors, since it is lost when such receptors are pharmacologically or genetically inactivated. This is in agreement with the finding of ghrelin-induced elevation of EC levels in the hypothalamus [20]. Importantly, also the effects of both peripherally and intracerebroventricularly administered ghrelin on liver, heart, and adipose tissue AMP kinase activity cannot be observed in CB1 knockout mice or in CB1 antagonist-treated mice. This suggests that also the metabolic effects of ghrelin in peripheral tissues, exerted via AMP kinase-mediated central or peripheral mechanisms, are partly due to elevation of ECS tone at CB1 receptors [29].

Both leptin and ghrelin are also deeply involved in the neural processing of the hedonic aspects of palatable food intake. Accordingly, ECS and CB1 activation also affect energy homeostasis by acting on the reward and reinforcement circuits of the mesolimbic system (reviewed in ref. 30), especially the NAc and ventral tegmental area (VTA), where the ECS is highly expressed and interacts with both dopaminergic and opiodergic pathways, resulting in preference for highly palatable food [31,32]. For example, Δ^9 -tetrahydrocannabinol increases sucrose-induced hedonic activity and dopamine release into the NAc [33], whereas CB1 antagonism reduces the increase of extracellular dopamine release induced in this nucleus by a novel highly palatable food [31]. Furthermore, CB1 and μ -opioid blockade synergize at reducing food intake and body weight in rodents [34,35]. As an example of findings linking the reward system and food intake through the ECS, fasting was found to raise endocannabinoid levels in the NAc,



and direct administration of the ECs 2-AG or AEA, or of synthetic compounds that inhibit EC inactivation in this area, increased food intake in a CB1-dependent manner [2,21]. EC signaling in the VTA, however, can also be associated with counter-rewarding stimuli. Synaptic strengthening of dopaminergic signaling in the VTA, and consequent phasic dopaminergic output, are associated with learned associations of cues with rewards [36]. Consistent with behavioral data demonstrating that intra-VTA insulin suppresses the salience of food-predicting cues, insulin reduces excitatory synaptic transmission onto dopamine neurons of the VTA [37]. This reduction in synaptic efficacy is long lasting and selective for excitatory inputs to the VTA. Insulin receptor activation leads to a postsynaptic activation of insulin receptor tyrosine kinase, Akt, and mammalian target of rapamycin signaling, which in turn increases synthesis of the EC 2-AG, leading to a retrograde inhibition of glutamate release via CB1 receptor activation. Interestingly, only one hour of access to sweetened high fat food occludes insulin induced long-term depression and increases EC signaling resulting in decreased presynaptic glutamate release. This effect correlates with elevated plasma insulin levels and reverses an hour after the sweetened high fat food exposure, when insulin levels are returned to baseline [37]. Together, insulin action in the VTA, either exogenously applied or endogenously induced by a caloric meal, transiently suppresses excitatory inputs (possibly relaying information about food-related cues) to VTA dopamine neurons via CB1 activation, thus providing an example of how EC signaling can also convey anorexigenic actions in extra-hypothalamic areas. Importantly, leptin also suppresses synaptic transmission at excitatory synapses onto dopamine neurons [38]. Although leptin can act postsynaptically on dopamine neurons to reduce firing rate [39], the hormone also acts presynaptically to inhibit glutamate release onto dopamine neurons via PI3K and Jak2 tyrosine kinase signaling [38]. Whether this effect undergoes negative feed-back via leptin-induced inhibition of EC signaling at these synapses remains to be established.

Possibly related to the role of ECs in the regulation of hedonic control of food intake also in humans, and to the contribution to such hedonic control of gastrointestinal hormones, it was reported that exposure and consumption of a favorite palatable food, as compared to normal food, by healthy volunteers is accompanied by elevated plasma 2-AG levels, which correlates positively with plasma ghrelin levels [40]. It is not known whether this phenomenon reflects a direct or indirect stimulatory action by palatable-food-enhanced ghrelin signaling on EC levels in the brain or peripheral tissues. It is also possible that this finding, particularly in relation to the post-consummatory elevation of 2-AG blood levels, is a consequence of a possible enhancement of small intestine EC levels after mouth exposure to fat, a phenomenon shown to occur in rats and to be due to stimulation of vagal fibers linking the mouth to the gut via the brainstem [41].

The role of sensory neurons in EC-mediated stimulation of food intake was also very recently highlighted by a study

demonstrating how activation by ECs of CB1 receptors in the olfactory cortex increases odor detection and food intake in fasted mice by decreasing the excitatory drive from olfactory cortex areas to the main olfactory bulb. Accordingly, CB1 receptors were shown to be abundantly expressed on axon terminals of centrifugal cortical glutamatergic neurons that project to inhibitory granule cells of the olfactory bulb. Thus, food deprivation-induced activation of the ECS links the feeling of hunger to stronger odor processing [42]. This elegant study extends previous observations [43] which had evidenced how 2-AG is synthesized in both olfactory receptor neurons and glia-like tentacular cells in larval *Xenopus laevis*, with levels that increase following food deprivation. The EC then controls odorant detection thresholds via CB1 activation in a way that hunger renders olfactory neurons more sensitive, thus positively influencing food-seeking behavior [43].

The nucleus of the solitary tract (NTS) is another brain structure that receives sensory inputs from the nodose ganglion, inputs that originate in the gut in response to ingested food. ECs in the hindbrain regulate gustatory neurotransmission initiated by palatable foods. Taste-related neural signals, including those associated with fatty and sweet foods, are transmitted from the oral cavity first to NST and then to the pontine parabrachial nucleus (PBN) in the brainstem. An overarching function of the ECS modulates the activity of central and peripheral neural pathways involved in the intake of sweet and fatty foods [41] (Fig. 1).

Among other brain areas, the NTS projects to the hypothalamus, thus forming a neural circuit that contributes to the autonomic regulation of food intake. Interestingly, CB1 receptors are expressed at the NTS and the nodose ganglion and they participate in the modulation of food intake by this circuit and are under the negative control of CCK signaling [44,45]. Finally, the central nervous system is involved in EC control of energy expenditure via the sympathetic nervous system, as shown by studies with selective deletion of CB1 receptors in central neurons, which confers resistance to high fat diet-induced obesity [46], or in both central and sympathetic neurons, which results in increased thermogenesis [47]. Accumulating evidence indicates a negative control of hypothalamic sympathetic outflow on lipogenesis through reduction of anandamide synthesis in adipose tissue [48]. Moreover, it is also been shown that hypothalamic CB1 receptors control energy expenditure through modulation of $\beta(3)$ -adrenergic receptor and uncoupling protein-1 in the brown adipose tissue since conditional mutant mice lacking hypothalamic CB1 receptors under a normocaloric, standard diet show not only decreased body weight gain over time, but also elevated energy expenditure, and increased $\beta(3)$ -adrenergic receptor and uncoupling protein-1 mRNA levels in the brown adipose tissue, in the absence of any effect on food intake [9]. These effects are required to induce thermogenesis in the brown adipose tissue and lipolysis in the white adipose tissue [49]. These data emphasize the key role of neuronal CB1 receptors in the

control of both central and peripheral energy homeostasis. However, they do not leave out the role of peripheral, non-neuronal CB1 receptors in the control of energy homeostasis.

3. Peripheral Endocannabinoid Regulation of Energy Homeostasis in a Nutshell, With Focus on Some Latest Discoveries

In addition to its role in modulating CNS processes involved in food intake and energy expenditure and in the crosstalk between the CNS and peripheral organs through the peripheral nervous system and hormones, the ECS is present in metabolically relevant peripheral tissues, that is the liver, adipose tissue, skeletal muscle, and endocrine pancreas, the normal physiology of which are modulated by ECs particularly via CB1 receptors.

CB1 receptors in the adipose tissue have been found to promote lipogenesis by several mechanisms, including facilitation of adipocyte differentiation and increased expression of adipogenic enzymes. Specifically, pharmacological activation of CB1 with WIN55,212-2 was found to increase lipoprotein lipase activity in primary adipocytes while rimonabant prevented this effect [50]. Rimonabant induced the expression of the adipocyte-derived hormone (adipokine), adiponectin, which is involved in insulin sensitivity and fatty acid breakdown [51]. In the adipose tissue, anandamide and 2-AG levels were found to be increased just before adipocyte differentiation, together with changes in the expression of enzymes involved in synthesis and degradation of these ECs [52], and adipogenic genes were induced after stimulation of CB1 receptors with HU-210 [53]. Interestingly, CB1 receptor antagonism by rimonabant and CB1 silencing by siRNA led to the “browning” of white adipose tissue, that is, to its transformation into a more thermogenic, mitochondria-enriched, and hence “brown-like” adipose tissue [54]. Noteworthy, the ECS in the brown adipose tissue is an important player in energy homeostasis. In fact, increased energy expenditure has been suggested to be more important than reduced food intake in the body-weight reducing effect of rimonabant [55] and, as mentioned before, chronic administration of rimonabant increases brown adipose tissue temperature and uncoupling protein-1 expression in this tissue, and activates brown adipose tissue in diet-induced obese mice [56]. Interestingly, differentiation was shown to be reduced, and apoptosis enhanced, in visceral adipocytes from CB1-receptor knockout mice as compared with wild-type controls, whereas CB1-null subcutaneous adipocytes showed an accelerated differentiation and a reduced rate of apoptosis. Furthermore, subcutaneous CB1-receptor knockout cells were more sensitive toward a conversion into a brown fat-like phenotype, with enhanced expression of uncoupling protein-1 and peroxisome proliferator-activated receptor gamma coactivator-1 α , and increased mitochondrial biogenesis and oxygen consumption [57]. These data, together with the previous observation of elevated and decreased endocannabinoid levels in visceral and

subcutaneous adipose tissue depots, respectively, in obese mice [52], suggest that CB1 activation in obesity may favor the formation of visceral adipose tissue—which contributes to obesity-induced inflammation and insulin resistance—at the expense of the subcutaneous adipose tissue—which instead plays a beneficial role in the protection from the metabolic consequences of excessive energy intake versus expenditure. The relevance of these findings to human obesity is underlined by the observation that a complete ECS is present in the human adipose tissue [58].

In the liver, CB1 receptor activation also induces lipogenesis, which is relevant to liver steatosis during obesity, and pharmacological CB1 activation in hepatocytes induces the expression of lipogenic enzymes [59] (Fig. 1). This phenomenon increases *de novo* fatty acid synthesis, whereas a high-fat diet was found to increase hepatic levels of the endocannabinoid AEA, CB1 density, and basal rates of fatty acid synthesis, with the latter being reduced by CB1 blockade [60]. Importantly, although liver-specific CB1 knockout mice develop obesity by high-fat diet exactly like wild-type mice, they were found to have less steatosis, hyperglycemia, dyslipidemia, and insulin and leptin resistance [61]. Recently, hepatosteatosis induced in mice by the activation of stearoyl-CoA-desaturase and subsequent excess formation of oleic and palmitoleic acid was suggested to be due to inhibition of FAAH, elevation of AEA levels, and overactivation of CB1 receptors [62].

CB1 receptors have also been found in muscle [63] and their pharmacological activation in the isolated soleus decreases both basal and insulin-stimulated glucose transport activity [64] (Fig. 1). Rimonabant directly improves glucose transport activity in a dose-dependent manner in this tissue [64,65]. Responsiveness of skeletal muscle to insulin seems to be mediated through modulation of PI3-kinase/PKB and Raf-MEK1/2-ERK1/2 signaling pathways which can be modulated by CB1 agonism [66].

The endocrine pancreas contains a full ECS, which has been suggested to be involved in glucose-stimulated insulin secretion and beta-cell mass expansion. CB1 and CB2 receptors as well as the ECS machinery have been found in islets [67,68]. Indeed, CB1 and CB2 expression in β -cells shows species-specific differences (reviewed in refs. 69,70). Specific activation of CB1 receptors in human islets increased insulin, glucagon, and somatostatin secretion, whereas CB2 activation decreased insulin secretion [71]. These effects seem to be mediated through modulation of glucose-induced intracellular calcium transients [72] and focal adhesion kinase [73]. Conversely, rimonabant was found to decrease insulin hypersecretion in islets from diabetic rats [74] and chronic treatment with rimonabant had a protective role against the development of hyperinsulinaemia, β -cell dysfunction, and islet disorganization in diabetic rats [75]. At least part of the benefits of CB1 antagonism in islets seems to be mediated through enhanced insulin receptor signaling leading to β -cell mass expansion [68]. However, a recent study showed how CB1 activation also in infiltrating macrophages in the pancreas from diabetic



Zucker rats activates the nucleotide-binding oligomerization domain-like receptor 3 (NLRP3) inflammasome, thus possibly contributing to β -cell loss in diabetes [76] (Fig. 1).

Taken together, these recent and more dated observations indicate, among other things, that, apart from modulating lipid metabolism, the ECS modulates glucose metabolism by promoting pancreatic hormonal secretion, which in turn favors glucose uptake by tissues such as the liver and white adipose tissue, leading to fatty acid synthesis and energy storage, or the skeletal muscle, leading to O_2 consumption during physical exercise. These effects are counteracted by the negative action of CB_1 stimulation on insulin sensitivity in the liver and skeletal muscle, which may prevail during obesity and other conditions leading to insulin resistance, thus causing a vicious cycle of more and more insulin production and less and less insulin action, with subsequent β -cell damage and type 2 diabetes, also via partly CB_1 -induced inflammation in the adipose tissue and pancreatic islets.

4. Regulation of EC Biosynthesis as a Possible Strategy to Control Energy Homeostasis: New Perspectives

As outlined in this article, strong alterations in EC levels are associated to unbalance of energy homeostasis in the liver, adipose tissue, pancreas, and skeletal muscle. These conditions contribute to hepatic steatosis, insulin resistance, adipocyte hypertrophy and inflammation, reduced glucose uptake, and oxygen consumption in the muscle and disrupted β -cell function [77] (Fig. 1). Apart from the first class of anti-obesity drugs based on CB_1 inverse agonists like rimonabant, and their contraindications which have imposed withdrawal from the market [78], a class of non-brain permeant CB_1 inverse agonists are currently being developed and tested in pre-clinical studies. Furthermore, inhibitors of 2-AG biosynthesis might have therapeutic applications to counteract positive energy homeostasis which underlies obesity and related metabolic disorders [79], possibly producing fewer side effects than CB_1 receptor inverse agonists [80–82]. In fact, O-5596, a prototypical inhibitor of 2-AG biosynthesis, was reported to transiently reduce the intake of palatable food in mice [83]. Recently, we reported the pharmacological characterization of several selective inhibitors of DAGL α [84]. In particular, we showed that acute i.p administration of the compound denoted as O-7460 dose-dependently inhibited food intake of high fat diet in mice causing an acute reduction of body weight by restoring the physiological levels of 2-AG in the hypothalamus and liver. Importantly, the effect of O-7460 on intake of high fat diet and body weight was not accompanied by overt actions on locomotor behavior or body temperature [85].

5. Concluding Remarks

The hypothalamus, the reward system, and the brainstem are major integrator centers for energy homeostasis that receive

inputs from both vagal afferents and hormonal signals and that, in turn, coordinate the function of peripheral metabolically relevant tissues by output signaling through sympathetic efferents. From a physiological point of view, EC signaling through CB_1 receptors has a general anabolic effect by: (i) promoting food intake through modulation of both pleasure and motivation to eat, (ii) re-directing lipid metabolism to pro-lipogenic and anti-lipolytic processes, and (iii) re-directing glucose metabolism to hepatic and adipose lipogenic actions [82]. Consequently, attenuating this pathway in people with a contemporary life style, where excessive energy storage versus expenditure within the body is a problem rather than an advantage, is expected to be beneficial to tackle obesity and related morbidities. First generation CB_1 receptor antagonists have been first launched on the market for the pharmacological management of these disorders and then discontinued because of unwanted psychiatric side effects [86]. More recent approaches for the counteraction of ECS dysregulation in obesity, metabolic syndrome, type 2 diabetes, and non-alcoholic steatosis are being tested with some success in animal models and include the use of peripherally restricted CB_1 antagonists, inhibitors of EC biosynthesis, and dietary interventions with $n - 3$ polyunsaturated fatty acids [82]. In particular, selective DAGL α inhibitors might be considered a useful pharmacological tool to further investigate the role played by 2-AG in the framework of endocannabinoid function in the control of palatable food intake and energy homeostasis. They might also be used to treat other conditions determined by pathologically elevated 2-AG levels, with hopefully less adverse effects than compounds like rimonabant.

Increasing evidence highlights the therapeutic effectiveness of the selective antagonism of CB_1 receptors in metabolically active peripheral tissue, such as the adipose tissue, liver, skeletal muscle, kidney, and pancreatic β -cells. Above all, the therapeutic efficacy of a peripherally restricted CB_1 antagonist in rodent models of the metabolic syndrome has been obtained using a novel CB_1 neutral antagonist (AM6545), an analog of rimonabant but more promising because of its inactivity in behavioral paradigms thought to be predictive of neuropsychiatric side effects in human [87]. AM6545 also sensitizes the adipose tissue to the lipolytic effects of endogenous leptin, as indicated by the ability of this compound to reduce body weight and adiposity in DIO but not in leptin-deficient *ob/ob* mice [87]. Moreover, the peripherally restricted CB_1 inverse agonist JD5037 resensitizes DIO mice to the central actions of leptin and restores hypothalamic leptin signaling in these mice by rapidly reversing their hyperleptinemia [88]. Therefore, peripherally acting CB_1 antagonists may represent a novel approach to reduce not only appetite and body weight, but also to restore leptin sensitivity.

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