ELSEVIER

Expression of the cannabinoid type 1 (CB_1) receptor in brain and peripheral tissues suggests that the metabolic effects of selective CB_1 receptor antagonism might involve the modulation of energy homeostasis and interactions between the CB_1 receptor and other neurohumoral pathways.

CB_1 receptor antagonism: biological basis for metabolic effects

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The endocannabinoid system (ECS) is a complex physiologic system that affects metabolic pathways. A dysregulated ECS has been demonstrated in animal models of obesity and the expression of the cannabinoid type 1 (CB_1) receptor in both brain and peripheral tissues suggests that selective antagonism at this receptor could target multiple tissues involved in metabolic homeostasis. In clinical trials with obese patients, treatment with the CB_1 receptor antagonist rimonabant was associated with clinically meaningful weight loss, as well as improved serum lipids and glycemic control. The biological basis for the metabolic effects of rimonabant (SR141716) appears to involve the modulation of metabolism through antagonism at a single receptor with several target organs.

Key resources

Websites

Endocannabinoid Research Group: http://www.icb.cnr.it/erg
Endocannabinoid System Network: http://www.endocannabinoid.net
The International Cannabinoid Research Society: http://www.cannabinoidsociety.org

Textbooks

The Cannabinoid Receptors (*The Receptors*), Patricia H. Reggio: Humana Press; 1st edition (August 2008), ISBN-10: 1588297128; ISBN-13: 978-1588297129
Cannabinoids (*Handbook of Experimental Pharmacology*), Roger G. Pertwee (Editor): Springer; 1st edition (April 29, 2005), ISBN-10: 354022565X, ISBN-13: 978-3540225652

The endocannabinoid system (ECS) is a complex physiologic system that affects multiple functions in the brain and other organs [1]. Seven transmembrane-domain cannabinoid receptors were cloned in the early 1990s, followed by the discovery of endogenous ligands, termed endocannabinoids [2,3]. The ECS comprises cannabinoid receptors, endocannabinoids and the enzymes involved in endocannabinoid synthesis and degradation [2,3]. The most-studied endocannabinoids are arachidonoyl ethanolamide (anandamide) and 2-AG [4,5]. Other newly proposed endocannabinoids have been described and their physiological role is under investigation [2]. Recently hemopressin, a novel bioactive peptide derived from the α1-chain of hemoglobin [6], was

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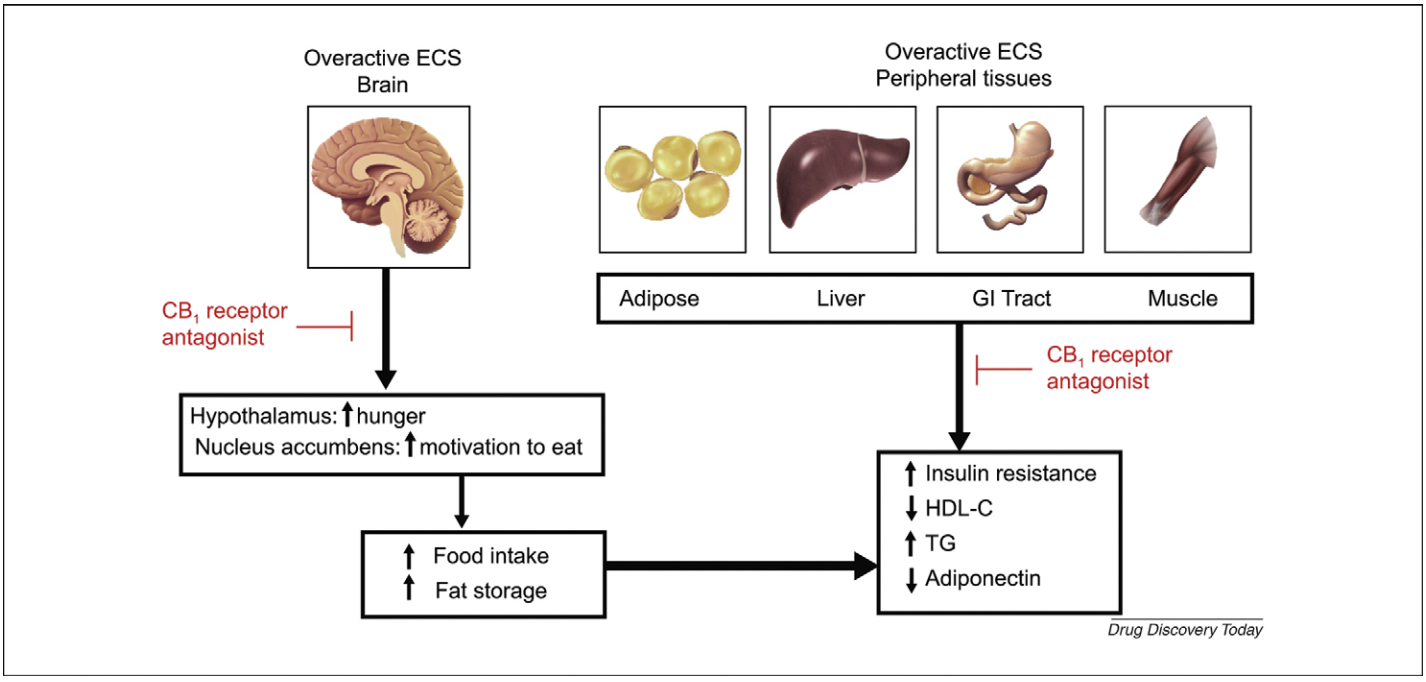

FIGURE 1

Hypothetical model for the metabolic effects of CB_1 receptor antagonism. Perturbations of the ECS might contribute to the etiology of obesity, insulin resistance, type 2 diabetes and dyslipidemia. CB_1 receptors are expressed in organs and tissues involved in the storage or metabolism of substrates. Evidence from cell culture has shown the effects of CB_1 receptor activation in adipocytes, hepatocytes, skeletal muscle myotubes and possibly, pancreatic β-cells. Evidence from animal studies has shown that CB_1 receptor blockade – both pharmacologic and genetic – attenuates diet-induced obesity, dyslipidemia, fatty liver and insulin resistance [1,8,35,37,115,116]. Both central and peripheral receptors might play a role in the regulation of food intake by the ECS [117]. Clinical data from the RIO trials have shown that CB_1 receptor antagonism (with rimonabant) reduces body weight, waist circumference and has favorable effects on dyslipidemia and glucose tolerance [9,10]. Adapted from Francischetti and de Abreu [118].

identified as a CB_1 receptor-selective antagonist [7]. Hemopressin also behaves as a CB_1 receptor inverse agonist, because it was shown to exert actions opposite to those of agonists in the apparent absence of endogenous agonists [7].

The ECS is expressed both in the brain and in the peripheral tissues and its stimulation increases food intake, promotes weight gain and metabolic pathways related to lipogenesis and impairs glucose tolerance in animal models [1,8]. Because the activity of the ECS might be increased in obese states, pharmacologic blockade of this system might represent a promising approach for the treatment of obesity and related metabolic disorders.

In large-scale clinical trials, attenuation of ECS activity with the selective cannabinoid type 1 (CB_1) receptor antagonist SR141716 (rimonabant) resulted in clinically significant weight loss and reduced waist circumference [9–12]. Additional effects of rimonabant treatment include improvements in serum lipids, fasting insulin and glycemic control in patients with type 2 diabetes. An overview of the metabolic effects of CB_1 receptor blockade is depicted in Fig. 1. Some of these effects might be independent of weight loss, suggesting direct peripheral metabolic effects of CB_1 receptor antagonism. After reviewing the biology of the ECS, this article describes potential mechanisms underlying the observed metabolic effects of CB_1 receptor antagonism.

Biology of the ECS

Endocannabinoids and endocannabinoid receptors

Endocannabinoids are derivatives of arachidonic acid [2]. Membrane depolarization of neurons or activation of certain receptors in many cells leads to the formation of anandamide and 2-AG from phospholipid precursors [2,13]. Unlike peptide or aminergic neurotransmitters, endocannabinoids are lipophilic neuromodulators and are not stored in synaptic vesicles [2]. Endocannabinoids appear to be produced 'on demand' and act on cells in a paracrine or autocrine manner [2,15].

The CB_1 receptor was first cloned from rat cerebral cortex, then from human brain and testis and then from mouse brain [3,15]. CB_1 receptors are expressed in a wide range of tissues, including peripheral tissues and organs involved in metabolism, such as adipose, skeletal muscle, liver, gastrointestinal (GI) tract and pancreas [16,8]. In the brain, the level of CB_1 receptor expression varies between neuronal subpopulations and brain regions; however, there is little correlation between the levels of expression and the receptor functional activity [3]. A second endocannabinoid receptor, the CB_2 receptor, is expressed in the spleen and tonsils, as well as in immune cells (B cells, monocytes and T cells), indicating a role in immune function, and might also be expressed in nervous tissue [16].

CB_1 receptor signal transduction and endocannabinoid catabolism

The CB_1 receptor is a member of the superfamily of G protein-coupled receptors (GPCRs) [16,17]. Activation of CB_1 receptors on nerve terminals inhibits both excitatory and inhibitory neurotransmission in many brain regions, including striatum, hippocampus, cerebellum, cortex, hypothalamus and nucleus accumbens, among others [18]. In neurons, CB_1 receptor stimulation is directly coupled to the inhibition of voltage-activated Ca^{2+}

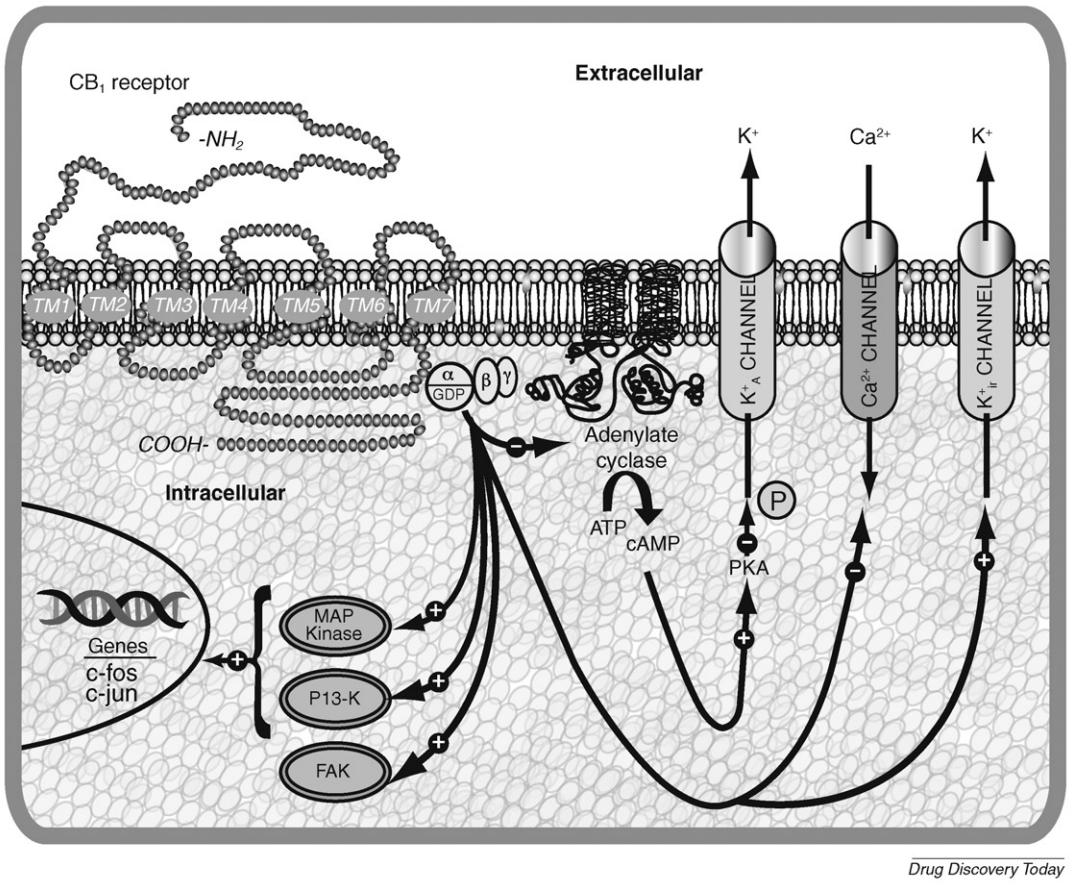

FIGURE 2

CB_1 receptor intracellular signaling cascades. Activation of the CB_1 receptor stimulates $G_{i/o}$ proteins, which leads to the inhibition of adenylate cyclase-mediated conversion of adenosine triphosphate (ATP) to cyclic AMP (cAMP). cAMP molecules can bind the regulatory subunits of protein kinase A (PKA) and cause the liberation of the catalytic subunits. Activated PKA can phosphorylate A-type potassium (K_A^+) channels, causing a decrease in current. Thus, CB_1 receptor activation results in the stimulation of K_A^+ channels. $G_{i/o}$ activated by the CB_1 receptor can also directly inhibit N- or P/Q-type Ca^{2+} channels and activate inwardly rectifying potassium (K_{ir}) channels. Activation of the CB_1 receptor can also stimulate several intracellular kinases, such as focal adhesion kinase (FAK), phosphatidyl inositol-3-kinase (PI3-K) and mitogen-activated protein kinase (MAP kinase). Stimulation of these or other protein kinases probably mediates the CB_1 receptor-induced expression of immediate early genes, such as the transcription factors c-fos and c-jun. Note that these events might not all occur in the same cell type. Reproduced with permission [119].

channels [3,19] and mediates retrograde signal transduction [13]. CB_1 receptor stimulation appears to activate inwardly rectifying K^+ channels, which decreases neuronal excitability. In peripheral tissues and neurons, CB_1 receptor activation inhibits adenylate cyclase with corresponding attenuation of the protein kinase A signaling cascade and stimulates mitogen-activated protein kinase pathways (Fig. 2) [3,19]. The type of signaling pathway affected by CB_1 receptor activation depends on the type of agonist used and the tissue or organ involved [3]. In fact, in some cell types, or even in the same cell type but from different species (as in the case of pancreatic β-cells), CB_1- or CB_2-receptor-induced stimulation or inhibition of intracellular Ca^{2+} might lead to stimulation or inhibition of insulin release [20–22]. In other cases, cannabinoid-mediated inhibition or stimulation of adenylate cyclase has been observed [3,16]. The stimulatory effect of the protein kinase A pathway on adenosine 5′-monophosphate-activated protein (AMP) kinase activity, together with the opposite effects of CB_1 receptor on protein kinase A signaling, might explain why cannabinoids stimulate AMP kinase activity in the hypothalamus and inhibit it in the liver and adipose tissue in rats [23]. The enzyme fatty-acid amide hydrolase (FAAH) catalyzes the hydrolysis of anandamide *in vivo* and, to some extent, also that of 2-AG [14,24–26]. A monoacylglycerol lipase is believed to play a role in the enzymatic hydrolysis of 2-AG [27]. Additional metabolic pathways for the endocannabinoids have also been described [28,29].

The ECS and metabolic homeostasis

The ECS and adiposity

The CB_1 receptor is an integral component of the neuronal pathways in the hypothalamus and hindbrain that control appetite, food intake and energy balance [1].

Following a stressful stimulus, ECS activation appears to promote, among other things, food ingestion, relaxation, pain reduction and extinction of aversive memories [30–32]. In this scenario, the ECS-induced drive to eat might be viewed as the need to

replenish energy stores to recover from one of the physiologic consequences of stress [30].

Studies in animal models have shown that the ECS influences adiposity, not only through centrally mediated appetitive mechanisms, but also through peripheral metabolic effects [33–39]. These preclinical studies suggest that stimulated CB_1 receptors promote fat storage and clinical studies demonstrate that the ECS in the adipose tissue might be dysregulated in human obesity, thus contributing to fat accumulation.

Feeding behavior and adiposity: mechanistic insights from the laboratory

Salamone *et al.* [40] showed that SR141716 treatment reduced the intake comparably in rats fed highly palatable diets or standard chow. In this study, adult male Sprague–Dawley rats were fed a high-fat diet (45% kcal from fat), a high-carbohydrate diet (67% kcal from carbohydrate) or a standard chow [40]. Rats received one injection of SR141716, at doses of 0.5, 1.0, 2.0 and 4.0 mg/kg in addition to vehicle, 30 min before the test session. SR141716 treatment was associated with significantly ($P < 0.05$) reduced food intake, which was comparable among rats fed the three different diets [40]. By contrast, Mathes *et al.* [41] showed that SR141716 treatment reduced the caloric intake but that the reduction was specific to a decrease in palatable food. In this study, adult female Sprague–Dawley rats received one injection of vehicle or SR141716 at 1.0 mg/kg 30 min before access to the sugar fat whip dessert. Compared with control, treatment with SR141716 was associated with significantly ($P < 0.05$) reduced caloric intake from the dessert. Consumption of the moist chow was not significantly different between the control and the SR141716 treatment groups. It is unclear what effect the study methodologies had on the outcomes. Additional studies are needed to clarify the qualitative effects of SR141716 on food intake.

The biological basis for the effects of the ECS on feeding behavior and adiposity appears to involve both central and peripheral targets. Numerous animal models of obesity demonstrate that treatment with CB_1 receptor antagonists reduces food intake and body weight gain [33–38]. The effects of CB_1 receptor antagonists are attributed specifically to endogenous CB_1 receptors; selective antagonism of the CB_1 receptor decreased food intake in wild-type mice, but had no effect on food intake in CB_1 receptor knockout mice [36].

CB_1 receptors might play a direct role in regulating energy expenditure. A recent study in rats demonstrated that CB_1 receptor antagonism with SR141716 was associated with increased energy expenditure. Compared with control animals, rats treated with 3 and 10 mg/kg rimonabant had an increase in O_2 consumption of 18% and 49%, respectively, after three hours [42]. There did not appear to be changes in the rate of carbohydrate and fat oxidation, and factors other than physical activity appeared to contribute to the increase in O_2 consumption [42].

There appears to be complex crosstalk between the ECS and leptin that regulates appetite-related neural circuits. Hypothalamic endocannabinoids might be under negative control by leptin, an adipocyte-derived hormone that inhibits orexigenic signaling in the hypothalamus. Genetically obese rats and mice with disrupted leptin signaling (Zucker *fa*/*fa* rats and *db*/*db* mice), as well as mice lacking leptin (*ob*/*ob* mice), have higher levels of endocannabinoids in the hypothalamus compared with wild-type animals (Fig. 3a) [43]. The influence of leptin on endocannabinoids appears to occur specifically in the hypothalamus, because levels of endocannabinoids in the cerebellum did not differ between *db*/*db* mice and wild-type mice [43]. Jo *et al.* [44] showed that endocannabinoids acting at CB_1 receptors mediate the suppression of inhibitory postsynaptic currents in perifornical lateral hypothalamic neurons, which results in the disinhibition of the release of melanin-concentrating hormone, an orexigenic mediator. Interestingly, leptin negatively controls this effect of the endocannabinoids (Fig. 3b).

Studies in diet-induced obese mice and CB_1 receptor knockout mice indicate that the ECS affects adiposity through both central effects on appetite and peripheral effects in adipose and possibly other tissues. In diet-induced obese mice, ten weeks of treatment with the CB_1 receptor antagonist SR141716 resulted in a sustained decrease in body weight, despite a reduction in food intake for only 14 days [37]. CB_1 receptor knockout mice exhibit decreased food intake, body weight and total fat mass compared to wild-type mice fed the same amount of food consumed by CB_1 receptor knockout mice (defined as 'pair-fed' mice). A significantly higher body weight was observed in 20-week-old, but not in 3-week-old, wild-type mice fed with the same amount of food as age-matched CB_1 receptor knockout mice, indicating that additional food intake-independent mechanisms contribute to the lean phenotype of adult CB_1 receptor knockout mice [39]. Importantly, CB_1 receptor knockout mice are resistant to diet-induced obesity [38,45]. Pair-feeding studies are important because they demonstrate that factors other than reduced food intake contribute to the lean phenotype of CB_1 receptor knockout mice. A pair-feeding study by Thornton-Jones *et al.* [46], however, suggested that the decreased body weight in SR141716-treated rats with diet-induced obesity was entirely accounted for by the reduction in food intake.

The GI tract is involved in the regulation of energy balance via neural and endocrine pathways [47]. In the GI tract, CB_1 receptors are present in neurons of the enteric nervous system and in sensory terminals of vagal and spinal neurons, activation of which has been shown to modulate several important aspects of nutrient processing, including gastric secretion, gastric emptying and intestinal motility [48]. Signals arising from the gut act in concert with central mechanisms to influence eating behavior [1]. Among the most important of these stills are the gut-derived hormones, such as cholecystokinin (CCK) and gastric leptin, which decrease food intake. Ghrelin, another gut-derived peptide, exerts an orexigenic effect [1]. These hormones function as satiety/hunger signals by triggering nerve impulses in sensory nerves traveling to the hindbrain [1]. They are also transported in the blood and are able to cross the blood–brain barrier, providing signals that are integrated in the hindbrain and the hypothalamus [1].

The vagus nerve, which connects the GI tract with the medulla and brainstem nuclei, might be a target through which the ECS modulates food intake [49]. The gut hormone CCK is secreted during a meal, and interacts with specific CCK receptors located on the afferent terminals of the vagus nerve [1]. CB_1 receptor mRNA levels on vagal afferent neurons projecting into the duodenum are decreased in rats fed *ad libitum*, while expression is increased when rats are food deprived [50]. Renewed feeding in previously fasted rats or the administration of CCK leads to decreased levels of CB_1

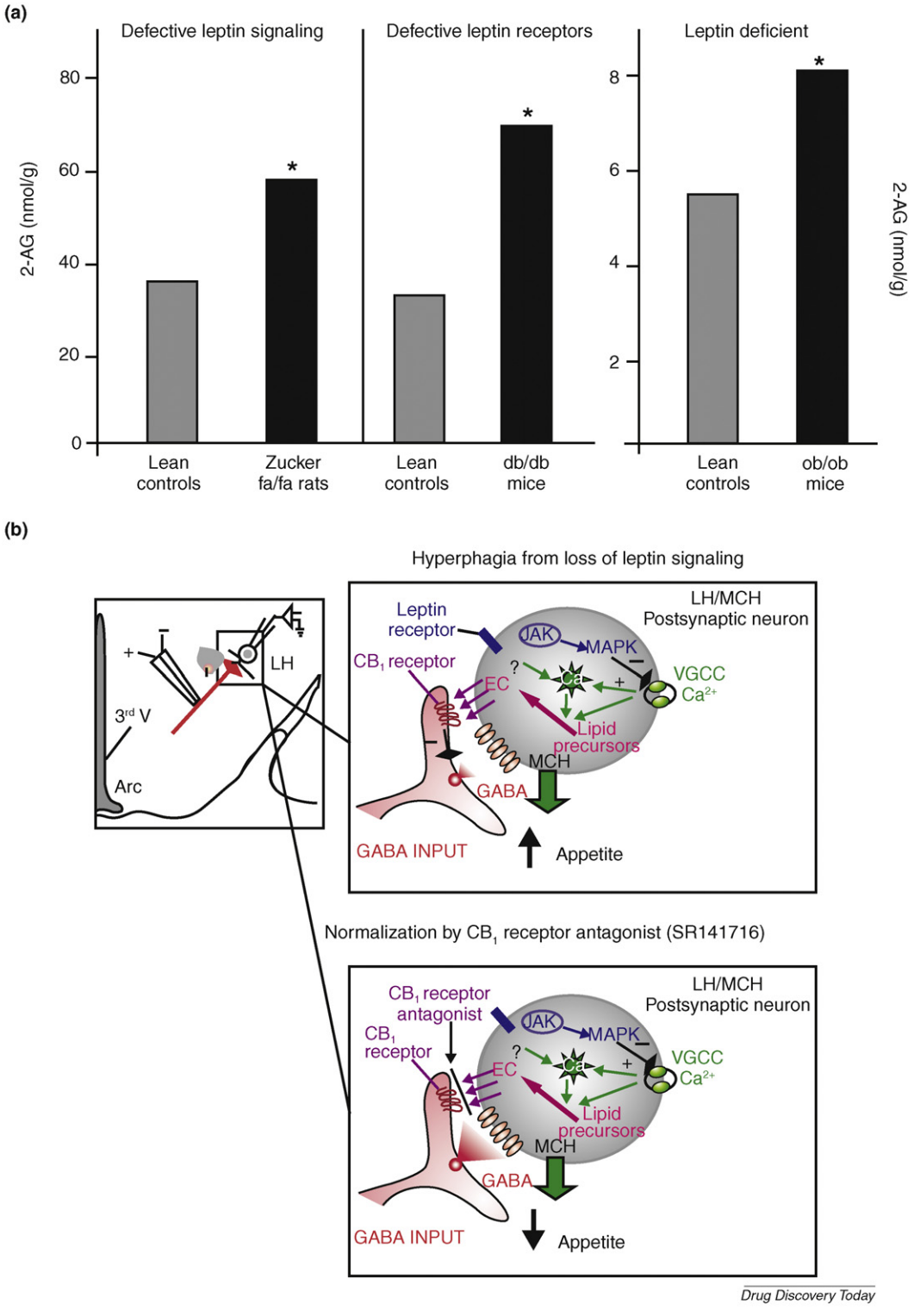

FIGURE 3

The ECS appears to be one of the targets through which leptin regulates energy intake and body weight. **(a)** Endocannabinoid levels in the hypothalamus are elevated in genetic models of obesity: Zucker *fa/fa* rats and *db/db* mice are obese and leptin resistant (left panel). A defect (in Zucker rats) or the genetic inactivation (in *db/db* mice) in the leptin receptor is responsible for the obese phenotype and susceptibility to diabetes of *db/db* mice (left and middle panels). The deficiency in leptin signaling in these genetically obese rodents is associated with increased levels of endocannabinoids in the hypothalamus (right panel) [43]. *$P < 0.05$. **(b)** Left: schematic of lateral hypothalamus (LH) and perifornical LH neurons. Melanin-concentrating hormone (MCH) neurons receive GABAergic inputs from diverse brain areas, including the nucleus accumbens/ventral striatum and the arcuate nucleus. The regulation of these GABAergic inhibitory tones to MCH neurons appears to be an important factor for controlling food intake and appetite. Right top:

receptor mRNA in the same vagal afferents [50], suggesting that reduced ECS activity might mediate the induction of satiety by CCK.

The ECS appears to be involved in mediating at least part of the orexigenic effect of ghrelin [51]. Blood levels of the gastric peptide ghrelin increase before a meal and decrease after the consumption of food, and exogenous administration of ghrelin increases food intake and adiposity [52]. In rats, the feeding-stimulatory effect of intracerebroventricular ghrelin infusion was blocked by pretreatment with a subanorectic dose of the CB_1 receptor antagonist SR141716 [51] and systemic administration of a CB_1 receptor antagonist suppressed circulating ghrelin levels [53]. Recently, Kola *et al.* [54] showed that ghrelin failed to induce an orexigenic effect in CB_1 receptor knockout mice. Moreover, ghrelin increased the endocannabinoid content of the hypothalamus in wild-type mice, an effect that was blocked by pretreatment with SR141716. No effect was observed in CB_1 receptor knockout mice. Thus, this study demonstrates that the ECS is necessary for the stimulatory effects of ghrelin on AMPK activity and food intake [54].

At the cellular level, the biological basis for the effects of CB_1 receptor antagonism on adiposity might involve the differentiation of preadipocytes. Support for the involvement of the ECS in adipose tissue lipogenesis comes from the finding that CB_1 receptor agonists increase lipoprotein lipase activity [39], stimulate peroxisome-proliferator-activated receptor γ (PPAR-γ) expression [20], enhance fatty-acid synthase expression [45] and inhibit AMPK activity [23] in adipocytes. Levels of the endocannabinoid 2-AG are increased in differentiated and hypertrophic mouse 3T3 F442A adipocytes compared with undifferentiated adipocytes [20] and there is an increase of anandamide synthesis, transport and binding efficiency of the CB_1 receptor during adipocyte differentiation [55]. However, the full functional implications of these data are still under study. Gary-Bobo *et al.* [56] demonstrated that CB_1 receptor antagonism inhibits the proliferation of 3T3 F442A preadipocytes. This might play a role in the reduced fat mass associated with CB_1 receptor antagonism in animals [56]. In mouse 3T3 F442A and human preadipocytes, stimulation of CB_1 receptors stimulates the expression of PPAR-γ and the amounts of lipid droplets, two markers of adipocyte differentiation [20,57]. Thus, CB_1 receptor activation might cooperate with PPAR-γ to promote early stages of adipocyte differentiation [58]. Moreover, these effects might mediate the effects of the ECS on metabolic homeostasis (reviewed in Pagano *et al.* [58]).

The ECS and human obesity

CB_1 receptors might play a role in regulating energy expenditure in humans. The effect of the CB_1 receptor antagonist taranabant on resting energy expenditure was measured in 17 overweight or obese subjects. Compared with placebo, resting energy expenditure two to five hours post-treatment with 12-mg taranabant was significantly increased [59]. The 12-mg dose of taranabant appeared to increase the rate of fat oxidation, as evidenced by a significant decrease in the mean respiratory quotient compared with placebo [59].

Given the local release and rapid degradation of endocannabinoids, the significance of circulating endocannabinoids is unclear, because they might reflect either endocannabinoid turnover in circulating blood cells, or the spill-over of endocannabinoids from peripheral organs. Nevertheless, several studies have noted the differences among patient subgroups in these levels. Circulating levels of anandamide and 2-AG are increased in obese, compared with lean, postmenopausal women [60] and plasma levels of anandamide were shown to be twofold higher in obese women with a binge-eating disorder than in nonobese healthy women or nonobese bulimic women [61]. These findings suggest a possible involvement of anandamide in the hedonic aspects of some eating disorders [61]. Matias *et al.* [20] found that visceral, but not subcutaneous, adipose tissue from obese patients contains significantly higher levels of 2-AG than the visceral fat from nonobese volunteers [20]. These data are supported by the findings from Bluher *et al.* [62] and Côté *et al.* [63], who showed that higher plasma 2-AG levels in obese men and women correlated with intra-abdominal, but not subcutaneous, adiposity.

The link between the dysfunctional ECS and human obesity might involve genetic polymorphisms. A relatively common missense polymorphism for the gene encoding the FAAH enzyme results in a functionally deficient protein (subjects with this polymorphism have approximately half the FAAH enzymatic activity of those subjects without the polymorphism) [64]. A population study showed that significantly more subjects with this FAAH genotype were overweight or obese than those of normal weight [64]. The median body mass index (BMI) for all subjects was significantly greater in the homozygous FAAH polymorphism genotype group compared with subjects with a heterozygous or normal genotype [64]. These data, however, were not confirmed in a second study of obese patients [65], indicating that the role of FAAH polymorphisms in obesity requires further investigation. If confirmed, the presence of a genetic malfunctioning of FAAH and the subsequent impaired degradation of endocannabinoids might underlie the permanently elevated peripheral levels of anandamide and/or 2-AG in obese patients. A study by Russo *et al.* [66] showed that single polymorphisms of the gene encoding the CB_1 receptor (*CNRI*) were associated with obesity-related phenotypes in men. These included a higher body weight and increased waist circumference and subscapular skinfold thickness [66].

Endocannabinoid level dysregulation might also be due to impaired leptin (Fig. 3a) and insulin action. Preclinical data support an important role for elevated leptin levels in the development of

proposed model for the mechanisms of ECS activity and modulation of GABAergic transmission in the perifornical LH neurons of the LH. The activation of presynaptic CB_1 receptors located on the GABA terminal decreases GABA release, thereby enhancing the net excitability of perifornical LH neurons, consistent with increased feeding behavior. Activated leptin receptors on perifornical LH neurons inhibit voltage-gated calcium currents (VGCC) via the activation of janus kinase 2 (JAK2) and MAP kinase. The consequent decrease in intracellular Ca^{2+} results in the less synthesis and release of endocannabinoids, and it decreases depolarization-induced suppression of inhibition. Perifornical LH neurons in leptin-deficient, obese mice (*ob/ob*) have larger VGCCs, consistent with increased endocannabinoid signaling, enhanced excitability and consequent hyperphagia. Right bottom: proposed model for the antiobesity effect of the CB_1 receptor antagonist SR141716. SR141716 would inhibit CB_1 receptors, antagonizing the elevated endocannabinoids from the MCH neuron. This would potentially normalize GABA release and inhibit MCH release, leading to decreased appetite.

leptin resistance and obesity [67]. In obesity, leptin loses the ability to inhibit energy intake and increase energy expenditure [68], and a central leptin insufficiency syndrome is thought to explain the adverse impact of deficient leptin signaling in the brain [69]. Such a syndrome might also cause elevation of endocannabinoid levels in the hypothalamus, and subsequent hyperphagia and body weight increase, although this hypothesis cannot be investigated in obese humans for obvious reasons. Insulin decreases endocannabinoid levels in insulinoma β-cells grown in low, but not in high, glucose [20] and stimulates FAAH expression (thereby possibly reducing endocannabinoid levels) in the subcutaneous adipose tissue of lean, but not obese, patients [70], where high levels of circulating 2-AG are also correlated with low levels of FAAH mRNA in visceral adipose tissue [62]. Therefore, elevated endocannabinoid levels might also be due to insulin resistance, particularly in peripheral organs.

The ECS and hepatic lipid metabolism

Biological basis for hepatic effects of CB_1 receptor antagonism: insights from the laboratory

Obesity in humans is associated with hepatic steatosis or fatty liver, which results from the accumulation of ectopic fat in hepatocytes [71]. Nonalcoholic steatohepatitis is an important cause of liver disease and there is strong evidence that the ECS also plays a role in hepatic fibrosis [72,73]. Indeed, preclinical studies support a hepatoprotective effect of CB_1 receptor antagonism in several pathological conditions. Histological analysis of the hepatic steatosis demonstrated that liver slices from genetically obese Zucker (*fa*/*fa*) rats treated with SR141716 were histologically comparable to those from lean rats, whereas there was still severe hepatic steatosis in the pair-fed obese (*fa*/*fa*) rats [34]. Moreover, diet-induced obese mice develop fatty liver after consuming a high-fat diet for three weeks, whereas CB_1 receptor knockout mice are resistant to the development of this disorder [45]. Hepatomegaly in obese Zucker (*fa*/*fa*) rats was characterized by a higher liver/body weight ratio (4.98 ± 0.15%) compared with that of lean littermates (3.50 ± 0.18%) [34]. Obese (*fa*/*fa*) rats treated with the CB_1 receptor antagonist SR141716 for eight weeks had a liver/body weight ratio comparable to that observed in lean rats. Notably, obese (*fa*/*fa*) rats pair-fed with SR141716-treated rats had only slight reduction in the liver/body weight ratio.

Other animal studies suggest that the ECS regulates hepatic lipogenesis by altering fatty-acid synthesis. For example, activated CB_1 receptors enhanced *de novo* lipogenesis in mouse hepatocytes [45]. A primary molecular pathway for hepatic lipogenesis involves the activation of the transcription factor sterol regulatory element-binding protein-1c (SREBP-1c) and its associated lipogenic enzymes, acetyl-CoA carboxylase-1 (ACC1) and fatty-acid synthase (FAS). Osei-Hyiaman *et al.* [45] demonstrated the role of the ECS in this pathway using rodent models of diet-induced obesity and CB_1 receptor knockout mice. Activation of the CB_1 receptor was associated with an increase in hepatic mRNA expression of transcription factor SREBP-1c and its target enzymes, ACC1 and FAS, in wild-type mice fed standard chow (Fig. 4) [45]. This was associated with a twofold increase in the rate of fatty-acid synthesis in the liver [45]. By contrast, fatty-acid synthesis did not increase in CB_1 receptor knockout mice or in mice pretreated with the CB_1 receptor antagonist SR141716, thus supporting a mechanistic role for the CB_1 receptor activation in promoting fatty-acid synthesis [45] (Box 1).

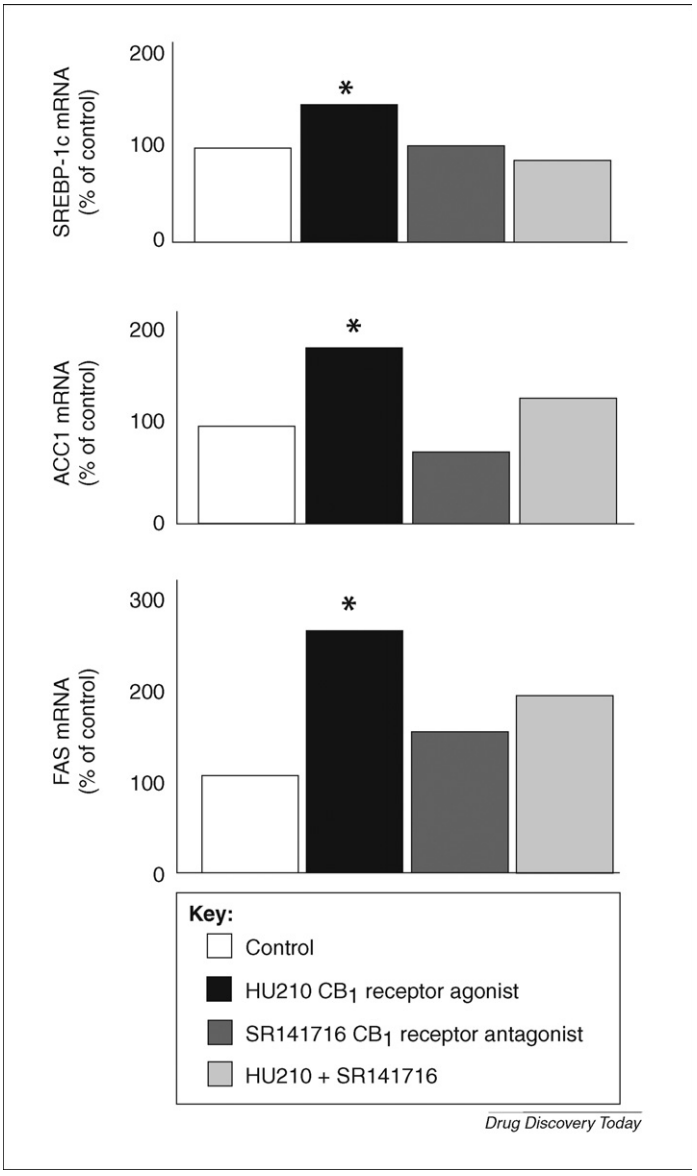

FIGURE 4

The CB_1 receptor modulates the expression of lipogenic enzymes in the liver. Mice were given intraperitoneal injections of vehicle, 20 ng/g HU210, 3 μg/g SR141716 or 20 ng/g HU210 plus 3 μg/g SR141716 one hour before sacrifice and removal of the liver. Activation of the CB_1 receptor with HU210 was associated with increased hepatic mRNA expression of transcription factor sterol regulatory element-binding protein (SREBP-1c, top panel) and the SREBP-1c target enzymes, acetyl-CoA carboxylase-1 (ACC1, bottom left) and fatty-acid synthase (FAS, bottom right). Relative mRNA levels were quantified by densitometry, corrected for 18S ribosomal RNA levels used as a loading control, and expressed as a percentage of the value measured in vehicle-treated controls. *$P < 0.05$ versus control. From Osei-Hyiaman *et al.* [45].

Hepatic CB_1 receptor stimulation *in vivo* contributes to the activation of the FAS lipogenic pathway, while CB_1 receptor blockade inhibits this effect [45]. Hepatic AMP kinase stimulates fatty-acid oxidation [74]. Kola *et al.* [23] demonstrated that cannabinoids inhibit AMP kinase activity in rat liver, although the involvement of CB_1 receptors was not demonstrated [23,75]. Although speculative, ECS activation might inhibit fatty-acid

BOX 1

Glossary of terms

2-Arachidonoylglycerol (n.) – 2-Arachidonoylglycerol (2-AG) is one of the first-discovered and best-studied endocannabinoids. 2-AG is a ligand for cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2). Generally, 2-AG is produced from the hydrolysis of 2-arachidonate-containing diacylglycerols.

Adenylyl cyclase (n.) – (Adenylate cyclase) Membrane-bound enzyme that catalyzes the formation of cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP).

Adiponectin (n.) – An adipocyte-derived hormone that has antidiabetic, anti-inflammatory and antiatherogenic properties. Decreased levels of plasma adiponectin are correlated with insulin resistance, dyslipidemia and cardiovascular disease. Adiponectin messenger ribonucleic acid and serum levels are decreased in obesity.

Agonist (n.) – A hormone, neurotransmitter, or a drug that binds to a specific site on a 'receptor' protein and stimulates a response.

Anandamide (n.) – Anandamide (*N*-arachidonoyl-ethanolamine) is the first-discovered and one of the best-studied endocannabinoids. Anandamide is a ligand for cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2); however, it is functionally more selective for the CB_1 receptor. Anandamide can interact with the transient receptor potential vanilloid type 1 (TRPV1) receptor, an ion channel found on sensory neurons, and can cause Ca^{2+} influx and neurotransmitter release [16]. Anandamide can also activate the nuclear receptors peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ although only at high micromolar concentrations [121,122].

Antagonist (n.) – A molecule that binds to a receptor but does not induce the necessary conformational change in the receptor to stimulate a cellular response.

Autocrine signaling (n.) – Cell signaling in which a cell secretes signaling molecules that act on the cell itself. Endocannabinoids act on cells in a paracrine or autocrine manner in peripheral tissues.

BMI (abbr.) (n.) – Body mass index. The BMI is used to express the degree of overweight. Calculated as the body weight in kilograms divided by the square of the height in meters [wt/(ht)^2]. A BMI > 30 kg/m^2 is synonymous with obesity, except in body builders and other athletes.

Cannabinoid (n.) – Cannabinoids are plant or synthetic compounds that might or might not act on the cannabinoid receptors. They include the herbal cannabinoids that occur uniquely in the plant *Cannabis sativa* and synthetic analogs produced by drug companies and research chemists.

Cannabis sativa (n.) – The cannabis plant (*Cannabis sativa*). This herb is also known as hemp or marijuana. Herbal cannabinoids occur uniquely in the cannabis plant. Tetrahydrocannabinol is the major psychotropic constituent.

cyclic AMP (cAMP) (n.) – Cyclic adenosine monophosphate. Nucleotide generated from ATP by adenylyl cyclase in response to the stimulation of many types of cell-surface receptors. cAMP activates cyclic-AMP-dependent kinase (protein kinase A, PKA).

CB_1 receptor (n.) – The cannabinoid receptor type 1 (CB_1 receptor) was the first cannabinoid receptor to be characterized. The CB_1 receptor is a member of the G protein-coupled receptor superfamily of cell-surface receptors. CB_1 receptors are present in many central and peripheral sites involved in the control of energy homeostasis and metabolism, including the brain, adipose tissue, liver, pancreas, gastrointestinal tract and skeletal muscle.

CB_2 receptor (n.) – The cannabinoid receptor type 2 (CB_2 receptor) was the second cannabinoid receptor to be characterized. The CB_2 receptor is a member of the G protein-coupled receptor superfamily of cell-surface receptors. CB_2 receptors are primarily expressed in the immune system but may also be expressed in the brain, pancreas, bone and other peripheral tissues.

Endocannabinoid system (n.) ECS – A complex physiological system that affects multiple metabolic pathways. It comprises CB receptors, their endogenous ligands (the endocannabinoids) and the proteins involved in endocannabinoid synthesis and inactivation, as well as the intracellular signaling pathways affected by endocannabinoids. Physiologically, the ECS plays a role in modulating energy balance, feeding behavior, hepatic lipogenesis and glucose homeostasis. The ECS also participates in numerous other physiologic processes including those related to immune response, neuroprotection, memory and learning, nociception (pain sense), fertility and bone turnover.

Endocannabinoids (n.) (pl.). – Endocannabinoids are endogenous agonists of cannabinoid receptors. Endocannabinoids are derived from long-chain polyunsaturated fatty-acids. The family of endogenous agonists for CB_1 receptors is larger than initially thought. The first-discovered and best-studied endocannabinoids are anandamide (*N*-arachidonoyl-ethanolamine) and 2-arachidonoylglycerol (2-AG). Newly proposed endocannabinoids are 2-arachidonyl-glyceryl ether (noladin, 2-AGE), *O*-arachidonoyl-ethanolamine (virodhamine) and *N*-arachidonoyl-dopamine (NADA). The physiological functions for these latter compounds have not yet been established. Anandamide, virodhamine and noladin have been shown to activate PPAR-α at high micromolar concentrations. Hemopressin was recently identified as an endogenous CB_1 receptor antagonist [8].

FAAH (abbr.) (n.) – Fatty-acid amide hydrolase (FAAH) catalyzes the hydrolysis of anandamide and, to a certain extent, 2-AG.

GPCRs (abbr.) (n.) – G protein-coupled receptors. The family of G proteins that interact with GPCRs is predominately the heterotrimeric G proteins, which consist of three different subunits, α, β and γ. When the G protein complex interacts with an active receptor, the α subunit exchanges its bound GDP (guanosine diphosphate) for GTP (guanosine triphospate) (hence the 'G' in G proteins), and dissociates from the β/γ dimer. CB_1 and CB_2 receptors primarily couple to G proteins of the inhibitory G protein ($G_{i/o}$) class. The α subunits from these G proteins inhibit adenylyl cyclase.

Hepatic steatosis (n.) – Yellow discoloration of the liver due to fat accumulation in the parenchymal cells.

Hyperphagia (n.) – Abnormally increased consumption of and appetite for food.

Leptin (n.) – A 16-kDa protein hormone that plays a key role in regulating energy intake and energy expenditure, including the regulation of appetite and metabolism.

MAPK (abbr.) (n.) – Mitogen-activated protein kinases (MAPK) are enzymes that perform crucial steps in relaying signals from the cell membrane to the nucleus. They are serine/threonine kinases, that is, they phosphorylate other proteins on serine/threonine residues. MAP kinases phosphorylate gene-regulatory proteins and other protein kinases. MAP kinases are usually activated only transiently in response to extracellular signals. Examples include extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinase (c-JNK) and p38 MAPK (p38). Activation of the CB_1 and CB_2 receptors can lead to activation of MAPK in some cell types.

Nucleus accumbens (n.) – A limbic forebrain area implicated in motivation. Dopaminergic axons project from the ventral tegmental area to the nucleus accumbens and the prefrontal cortex. This pathway is activated by the expectation of rewarding stimuli and by stressful or aversive stimuli.

Orexigenic (adj.) – Increasing or stimulating the appetite.

Oleylethanolamide (OEA) (n.) – A naturally occurring lipid that is structurally related to anandamide but does not bind

cannabinoid receptors. OEA induces satiety by binding to PPAR-α [123].
Pair-feeding (adv.) – Feeding a group of animals or subjects the same amount and composition of food as a test group of animals or subjects.
Paracrine signaling (n.) – Short-range cell–cell communication via secreted signaling molecules that act on adjacent cells. Endocannabinoids act on cells in a paracrine or autocrine manner in central and peripheral tissues.
PPARs (abbr.) (n.) **Peroxisome proliferator-activated receptors** – Nuclear transcription factors comprising different isoforms: PPAR-α, PPAR-γ and PPAR-δ. Regulation of PPAR target genes is involved in energy homeostasis, adipocyte differentiation and inflammation [58].
PKA (abbr.) (n.) – Protein kinase A (cyclic AMP-dependent protein kinase). Enzyme that phosphorylates target proteins in response to a rise in intracellular cyclic AMP.
Polymorphism (n.) – Many different alleles of a gene, none of which is predominant in the population.
Rimonabant (n.) – Rimonabant is a selective CB_1 receptor antagonist. The chemical name is *N*-piperino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide.
Retrograde signaling (n.) – The communication by signaling molecules derived from postsynaptic and delivered to presynaptic structures (opposite to the direction of travel of conventional neurotransmitters).
SREBP-1c (abbr.) (n.) – Sterol regulatory element-binding protein-1c is a transcription factor. A primary molecular pathway for hepatic lipogenesis involves the activation of SREBP-1c.
Subcutaneous adipose tissue (n.) – Adipose tissue between the fascia of skin and muscle.
Visceral adipose tissue (n.) – Adipose tissue surrounding the organs, or viscera, in the thorax, abdomen and pelvis. There is a strong, positive correlation between the amount of visceral adipose tissue and the health risks of obesity.

oxidation and stimulate *de novo* lipogenesis in the liver. CB_1 receptor antagonism with SR141716 was shown to inhibit the progression of fibrosis in a mouse model of chronic liver injury [76]. The CB_2 receptor, however, might instead be involved in hepatic antifibrogenic pathways [77]. Siegmund *et al.* [78] showed that 2-AG might produce antifibrogenic effects in the liver by inducing cell death in activated rat and human hepatic stellate cells, but not in hepatocytes. The 2-AG-induced cell death was, however, independent of CB_1 and CB_2 receptors [78]. Taken together, these studies suggest that the ECS might play a role in obesity-induced nonalcoholic fatty liver disease, but additional studies are needed to clarify the role of CB_1 and CB_2 receptors.

Chronic alcohol use can lead to the development of fatty liver, which can progress into hepatic steatosis and cirrhosis. Recently, Jeong *et al.* [79] demonstrated that the steatotic effects of ethanol (ETOH) are locally mediated via hepatic CB_1 receptors [79]. CB_1 receptor knockout mice were pair-fed with wild-type mice and then both groups of mice were fed ETOH. Wild-type but not CB_1 receptor knockout mice developed fatty liver and hepatocellular damage. Jeong *et al.* [79] also generated transgenic mice that had hepatocyte-specific deletion of the CB_1 receptor. These liver-specific CB_1 receptor knockout mice lack CB_1 receptors in hepatocytes but express normal CB_1 receptor levels in other tissues. Interestingly, the liver-specific CB_1 receptor knockout mice were resistant to ETOH-induced fatty liver. This study suggests that hepatic CB_1 receptors are involved in the development of ETOH-induced hepatic steatosis and that treatment with CB_1 receptor antagonists might represent a treatment strategy [79]. Importantly, a similar conclusion was reached, again by using hepatocyte-specific CB_1 receptor knockout mice, when examining the effect of CB_1 receptors in hepatocytes in diet-induced hepatic steatosis in mice, a model of nonalcoholic fatty liver disease [80].

The ECS and dyslipidemia/dyslipoproteinemia

Some lipid-profile abnormalities associated with diet-induced obesity are improved by CB_1 receptor antagonism. When diet-induced obese mice were treated with SR141716, serum triglycerides (TGs) and low-density lipoprotein cholesterol (LDL-C) were significantly reduced. Although high-density lipoprotein cholesterol (HDL-C) was not altered, the HDL-C/LDL-C ratio was significantly increased [37]. Thus, the ECS might be involved in the lipid and lipoprotein abnormalities associated with obesity. It is possible that elevated hepatic fatty-acid synthesis caused, among other things, by the overactive ECS in the liver, in concert with increased free fatty-acid release from the adipose tissue and increased dietary fatty-acid, participates in elevated hepatic TG synthesis and contributes to elevating very low-density lipoprotein cholesterol levels and, hence, to reducing HDL-C levels. This possibility would explain why blood 2-AG levels correlate positively with high TG levels and low HDL-C in obese male patients [63].

The ECS and glucose homeostasis

The ECS and glucose homeostasis: mechanistic insights from the laboratory

Glucose homeostasis is mediated in part by metabolic interactions among the pancreas, liver, adipose tissue, skeletal muscle and GI tract, all of which express the CB_1 receptor. Both CB_1 and CB_2 receptors are expressed in intact pancreatic islets of Langerhans isolated from mice [21], with CB_1 receptor expression being most abundant in the glucagon-producing α-cells and CB_2 receptor expression in both α-cells and the insulin-producing β-cells [21,81]. CB_1 and CB_2 receptor proteins were also detected in rat pancreatic β- and non-β-cells [22]. The implication of these findings for human islet and β-cell CB_1 and CB_2 receptor expression is unclear. Activation of CB_1 receptors *in vitro* appears to stimulate insulin secretion from rat-derived insulinoma β-cells [20,82]. When RIN-m5F insulin-secreting cells were stimulated with CB_1 receptor agonists, there was a significant potentiation of glucose-stimulated insulin secretion from the cells. Although the CB_1 receptor antagonists did not inhibit insulin secretion, they blocked the effect of the agonists. By contrast, in mouse β-cells it is CB_2 receptor stimulation that inhibits, rather than stimulates, insulin release [21]. *In vivo* data obtained in lean rats have shown that CB_1 receptor stimulation inhibits and CB_2 receptor stimulation enhances glucose clearance following oral administration of glucose, whereas CB_1 and CB_2 receptor blockades exert the opposite effects [22]. In view of the discrepant results on the effects of CB_1 and CB_2 agonists on insulin release, however, it is not clear whether these effects are due to actions at the level of insulin release from the β-cell, or of insulin sensitivity in the liver and the skeletal muscle.

Data from a cell culture study suggest that a high glucose concentration is associated with elevated endocannabinoid levels.

When RIN-m5F rat insulinoma β-pancreatic cells were cultured in medium containing concentrations of glucose that were minimal (13 mM) or high (25 mM) for this type of cell and then stimulated with a one-hour pulse of 33 mM glucose, anandamide and 2-AG were increased [20]. When cells were cultured in 13 mM glucose, the effect of 33 mM glucose was abolished when cells were stimulated with both 33 mM glucose and 100 nM insulin. Notably, insulin failed to inhibit the 33 mM glucose-induced endocannabinoid elevation in RIN-m5F cells cultured in 25 mM glucose [20]. These data suggest that chronic hyperglycemia might lead to elevated levels of anandamide and 2-AG, reflecting an increase in ECS activity. The increased ECS activity with severe hyperglycemia, in turn, might be associated with dysregulated insulin secretion.

Animal models of obesity support the clinical potential for CB_1 receptor antagonism to affect glucose homeostasis favorably [38]. Wild-type and CB_1 receptor knockout mice fed a high-fat diet have increases in fasting glycemia. The glucose-lowering effect of an intraperitoneal insulin injection was reduced in the wild-type mice. In CB_1 receptor knockout mice, the glucose-lowering effect of an intraperitoneal insulin injection was the same as in control mice fed standard chow [83]. Thus, CB_1 receptor knockout mice fed a high-fat diet did not develop the insulin resistance that was observed in the wild-type mice. In addition, the CB_1 receptor knockout mice maintained their lean phenotype, despite consuming a high-fat diet. Additional studies are needed to determine if protection from insulin resistance is independent of the lean phenotype in the CB_1 receptor knockout mouse fed a high-fat diet.

Animal studies also demonstrate that selective antagonism of the CB_1 receptor with SR141716 ameliorates abnormalities in glucose metabolism associated with diet-induced obesity [83]. When obese mice fed a high-fat diet were treated with SR141716 for five weeks, there was a transient reduction of food intake (48% on week one), but a marked and sustained reduction of body weight (20%). Notably, fasting insulin and glucose levels of the high-fat-fed mice treated with SR141716 were reduced to the levels observed in the mice fed standard chow, whereas the nontreated mice fed the high-fat diet had elevated fasting insulin and glucose levels [83].

Studies in mice suggest that the modulation of glycemic control by the ECS might be mediated, in part, by effects on skeletal muscle. Liu *et al.* [84] showed that the rate of glucose uptake by isolated soleus muscle was significantly increased in mice treated with SR141716 for seven days compared with vehicle-treated mice. It is unclear, however, whether this effect was independent of changes in body weight.

A role of the ECS in glucose homeostasis is also supported by molecular studies. For example, CB_1 receptor antagonism is associated with increased expression of genes involved in glucose metabolism. Treatment with the CB_1 receptor antagonist SR141716 enhanced the expression of genes that are the crucial regulators of glucose metabolism in diet-induced obese mice [20,85]. Global gene expression analyses demonstrated that SR141716 enhanced the expression of four of the eight glycolytic enzymes found in adipose tissue: phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase and β-enolase [85].

Finally, Osei-Hyiaman *et al.* [80] recently showed that the impaired glucose tolerance and insulin sensitivity typically observed in diet-induced obese mice can be antagonized by simply deleting CB_1 receptor expression in hepatocytes, thus underlying again the importance of hepatic CB_1 receptors in determining insulin resistance and glucose intolerance.

Human glucose metabolism and the ECS

Glucose homeostasis is regulated by peripheral tissues that express the CB_1 receptor, including pancreas, liver, adipose and skeletal muscle tissue [8]. Recent studies have shown that (1) the CB_1 receptor stimulation is coupled to enhanced insulin and glucagon release from human pancreatic β- and α-cells, although most CB_1 receptors seem to be present in α-cells [22], as previously shown for the mouse and rat [21,22] and (2) the CB_1 receptor antagonism is coupled to enhanced AMP kinase α1 expression in human skeletal muscle myotubes in culture and, therefore, to potentially higher insulin sensitivity and glucose uptake and oxidation [86]. These findings might indicate that CB_1 activation both contributes to insulin resistance at the level of the skeletal muscle (but not of the adipose tissue, where increased insulin sensitivity might contribute to *de novo* lipogenesis; see Pagano *et al.* [57]) and attempts to compensate this effect by increasing insulin release. The mechanisms through which CB_1 activation modulates insulin release, however, are yet to be established.

Matias *et al.* [20] showed that overweight subjects with type 2 diabetes or hyperglycemia had significantly higher blood endocannabinoid levels compared with age- and BMI-matched normoglycemic subjects, whereas reduced blood endocannabinoid levels instead accompany the transient hyperglycemia that follows a meal in lean subjects. This finding suggests that regulation of blood endocannabinoid levels might be disrupted in the presence of chronic hyperglycemia [20]. Of particular interest for future studies is to determine whether elevated endocannabinoid levels in visceral fat, and the subsequent increased lipogenesis and the capability of this tissue to release fatty-acids, are related to the insulin resistance induced by free fatty-acids in peripheral tissues [87], particularly in the liver. A hypothetical model of the biological basis for the glycemic effects of CB_1 receptor antagonism is shown in Fig. 5.

CB_1 receptor antagonism and adiponectin: mechanistic insights

Adiponectin is an adipocyte-derived hormone that has important regulatory roles in lipid and glucose metabolism [88]. Adiponectin inhibits obesity, dyslipidemia and insulin resistance: low levels of adiponectin are associated with abdominal obesity, insulin resistance and dyslipidemia [89,90] and polymorphisms in the gene coding for adiponectin are associated with obesity and insulin resistance [89]. It is plausible that changes in adiponectin might be related to ECS effects on lipid and glucose metabolism. Clinically, adiponectin levels rise when insulin sensitivity improves, such as after weight loss or treatment with insulin-sensitizing drugs [89]. Animal studies demonstrate that adiponectin can facilitate the actions of insulin on muscle and liver, and might also act in the central nervous system to facilitate glucose disposal and increase total energy oxidation [88,89]. Studies in mice show that intracerebroventricular administration of adiponectin is associated with reduced body weight and favorable changes in glucose metabolism [88]. CB_1 receptor stimulation appears to decrease adiponectin

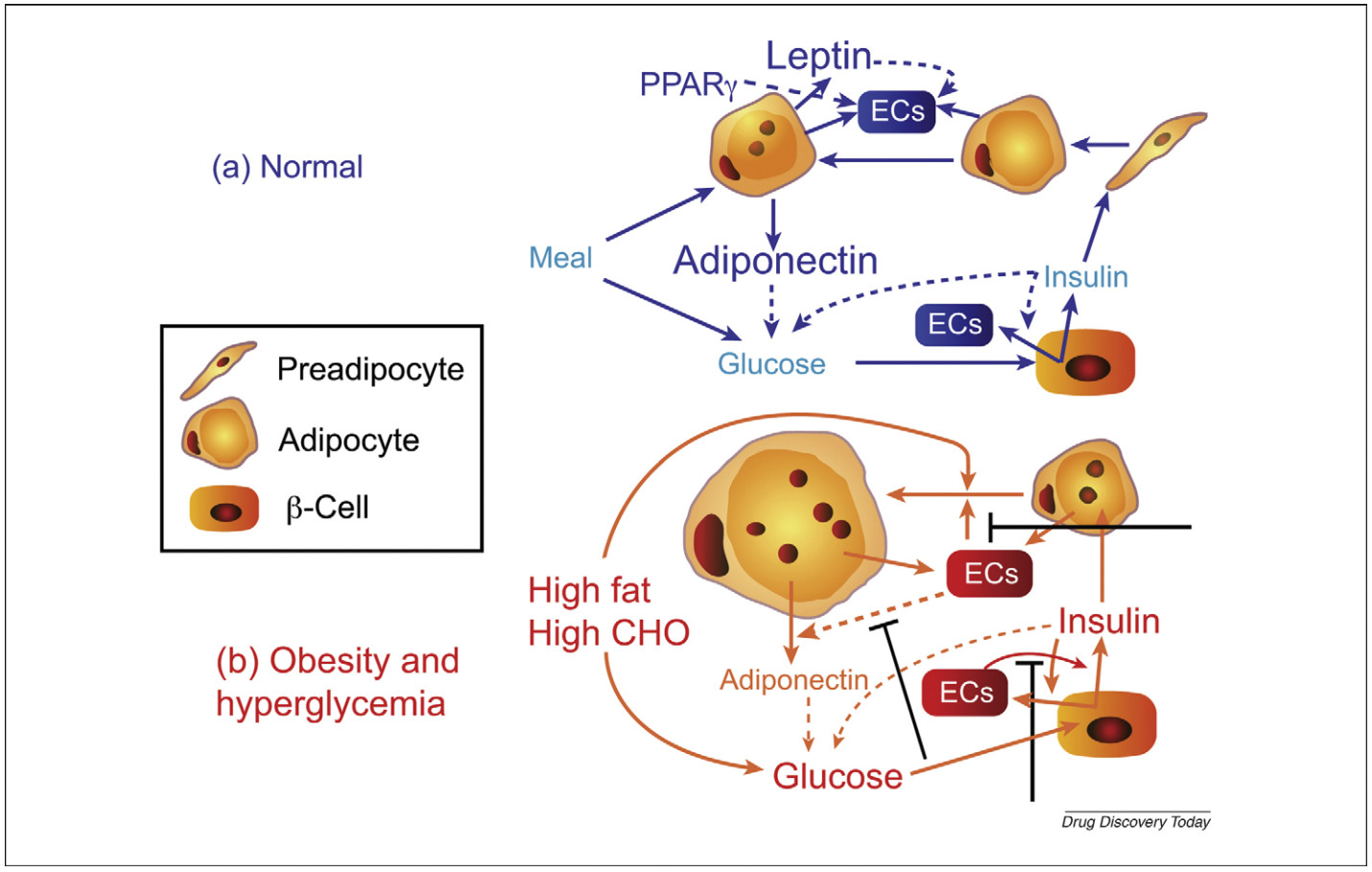

FIGURE 5

Hypothetical model of the biological basis for the antilipogenic proadiponectinemic and hyperinsulinemia-reducing effects of CB_1 receptor blockade in the adipose tissue. **(a)** Postprandial increases in serum glucose cause pancreatic β-cells to release insulin. Adipocytes are targeted by some of this insulin, which can induce cell differentiation and promote lipogenesis. Adiponectin regulates insulinemia and glucose homeostasis. Endocannabinoids (ECs) produced by pancreatic β-cells might be involved in insulin release but are negatively regulated by insulin. By contrast, ECs produced during adipogenesis contribute to lipogenesis and adipocyte differentiation but are negatively regulated by leptin and peroxisome-proliferator-activated receptor γ (PPAR-γ). **(b)** In obesity and prediabetes, hyperglycemia-induced insulin stimulates β-cells to produce higher levels of ECs. Increasing amounts of ECs and insulin are produced from β-cells, which might become hypertrophic. Excess insulin and dietary fats accelerate adipogenesis. This process is facilitated by adipocyte ECs, which inhibit adiponectin expression and thus, cause further hyperglycemia and eventually, insulin resistance and impaired glucose uptake. Arrows, activation; dashed arrows, inhibition; T-bar, potential sites of action for CB_1 receptor antagonist. Adapted from Matias *et al.* [20].

expression in mouse 3T3 F44A adipocytes [20] and CB_1 receptor blockade with SR141716 increases adiponectin levels in both diet-induced obese mice [37] and genetically obese rats [35]. Although these changes in adiponectin levels are associated with favorable changes in serum insulin and glucose levels [35,37], it is unclear whether the increased adiponectin levels were independent of weight loss in animals treated with SR141716, and, therefore, possibly due to direct effects occurring in the adipose tissue (Fig. 5). *In vitro* treatment of mouse adipocyte cells with SR141716 is in fact associated with significantly increased levels of adiponectin mRNA compared with control cells [20,35]. Lofgren *et al.* [91] determined the relationship between the expression of the CB_1 receptor gene (*CNR1*) and the levels of the adipocyte-derived hormone adiponectin. Subcutaneous fat biopsies were obtained from 96 obese and nonobese subjects and omental fat biopsies were obtained from 82 obese and nonobese subjects. There was no association of human adipose tissue *CNR1* mRNA expression with the expression of the adiponectin gene. In addition, there was no association of human adipose tissue *CNR1* mRNA expression with circulating adiponectin levels or adipose tissue adiponectin secretion [91]. Nevertheless, although CB_1 receptor gene expression in the human adipose tissue does not correlate with adiponectin gene expression, this finding does not preclude the existence of a tonic negative control by endocannabinoids on adiponectin levels in human adipocytes via CB_1 receptors. This would be similar to that shown to occur in mouse 3T3 adipocytes [20] (Fig. 5).

Pharmacological considerations on CB_1 receptor blockade

Although CB_1 receptor knockout mice are widely used as a means to understand the role of the ECS in metabolism, assess the specificity of pharmacological tools and, as discussed here, identify the biological bases of the metabolic effects of CB_1 receptor blockade, a clear distinction needs to be made between genetic and pharmacologic inactivation of CB_1 receptors. In fact, the former causes, in homozygotes, 100% blockade of the receptor, whereas antagonism with CB_1 antagonists/inverse agonists does

not saturate all receptors, even at doses that are maximally efficacious in clinical studies [92]. In fact, data from a human positron emission tomography brain imaging study suggest that when the CB_1 receptor inverse agonist taranabant is dosed in a therapeutic fashion, the compound occupies approximately 40% of brain CB_1 receptors [92]. Furthermore, pharmacologically, most CB_1 receptor antagonists developed to date demonstrate inverse agonism [40]; that is, they are capable of reducing the intrinsic tone produced by the CB_1 receptor also in the absence of exogenous agonist [40]. Indeed, a model has been proposed by Bouaboula *et al.*, in which rimonabant induces a receptor state that inhibits the putative endocannabinoid-independent coupling of CB_1 to the G protein [93], whereas a neutral antagonist would simply displace the agonist from the receptor and would only exert an effect in the presence of endogenous agonists [40]. Based on data from Chinese hamster ovary (CHO) cells transfected with the human CB_1 receptor, Bouaboula *et al.* proposed that CB_1 inverse agonists stabilize the complex between the GPCR and an inactive GDP-bound G protein [93]. Additional studies are needed to determine whether rimonabant inverse agonism is relevant for its pharmacological effects and/or potential adverse effects *in vivo*. Of interest is a recent study on the drug LH-21, a neutral cannabinoid receptor antagonist with a poor penetration rate into the central nervous system [94]. This study showed that chronic administration of LH-21 (3 mg/kg) in Zucker rats reduced feeding but did not improve hypertriglyceridemia or hypercholesterolemia, nor did it reduce liver fat deposits. These results might suggest that the inverse agonism and/or the antagonism of central CB_1 receptors might be necessary for the metabolic benefits of CB_1 receptor blockade. However, a more recent study showed that most of the metabolic effects of LH-21 might not be due to interaction with CB_1 receptors, because they persist in CB_1 receptor knockout mice [95]. Thus, LH-21 might not be the best tool to analyze the effects of neutral CB_1 receptor antagonism on unbalanced energy homeostasis.

Metabolic effects of CB_1 receptor antagonism in humans

Obesity

The international Rimonabant in Obesity (RIO) program evaluated the efficacy and safety of rimonabant in four Phase 3 randomized trials in which more than 6000 overweight or obese subjects received double-blind treatment with rimonabant 5 or 20 mg/d or placebo with diet and/or lifestyle modification therapy for one or two years. These trials consistently found that one year of treatment with rimonabant (20 mg/d) significantly increased weight loss (Table 1) and reduced waist circumference, compared with diet or lifestyle therapy alone [9–12]. In the RIO-North America study, subjects who continued to receive rimonabant (20 mg/d) during Year 2 maintained their weight loss, while those who received rimonabant (20 mg/d) during Year 1 and were switched to placebo during Year 2 regained weight back to the baseline level [11].

Results of a double-blind, placebo-controlled 12-week dose range-finding study were published recently for the CB_1 receptor inverse agonist taranabant [59]. Three hundred and fifty-eight obese or overweight adult subjects completed the 12-week study [59]. Treatment with taranabant significantly increased weight loss and reduced waist circumference at all evaluated doses (0.5, 2, 4 and 6 mg/d) compared with placebo. These effects were associated with a clinically small, but statistically significant increase in resting energy expenditure (6% increase [95% CI 1.01–1.10; $P = 0.011$]) following treatment with 12 mg taranabant (as measured by indirect calorimetry in 17 subjects), compared with placebo.

TABLE 1

Effect of rimonabant 20 mg on body weight among RIO studies.

Trial	Mean placebo subtracted one year weight loss (kg)
RIO-Europe	4.7
RIO-Lipids	5.4
RIO-North America	4.7
RIO-Diabetes	3.9

The effect of rimonabant 20 mg on body weight was similar among the RIO studies, and was statistically significant compared with weight loss observed with placebo. The average placebo-subtracted changes from baseline body weight ranged from 3.9 to 5.4 kg. Weight loss with 5 mg/d of rimonabant was not different from placebo. Adapted from Gadde and Allison [120].

Lipid profiles

The RIO-Lipids trial examined the effects of rimonabant on metabolic risk factors, including adiponectin levels [10]. This trial enrolled 1036 men and women without diabetes who had untreated dyslipidemia and a BMI between 27 and 40 kg/m^2. Compared with the placebo group, subjects treated with rimonabant (20 mg/d for one year) had significant weight loss, an increase in HDL-C and the reduction in plasma TGs. After adjustment for body weight loss, rimonabant 20 mg appeared to have a direct effect on increasing HDL-C and adiponectin and reducing TGs due to direct CB_1 receptor antagonism on peripheral tissues [96]. Regression analysis of change in HDL-C, triglycerides and adiponectin versus body weight at one year by analysis of covariance suggested that 45–57% of the effects of rimonabant 20 mg/d could not be explained by the observed weight loss. However, human pair-feeding studies, or clinical 'head-to-head' comparisons with other antiobesity agents with different mechanisms of action, are needed to confirm the degree to which these effects are independent of weight loss, and perhaps also to confirm or rule out a direct role of CB_1 receptor antagonism at increasing adiponectin levels in obese humans.

Indeed, other clinical trials of pharmacotherapy for weight loss demonstrated changes in some metabolic parameters. Treatment with orlistat was associated with the reductions of serum lipid levels, particularly of LDL-C [97]. The reduction in LDL-C levels appears to be greater than can be accounted for by a decrease in body weight; this reduction is presumably due to the fecal fat loss induced by the drug [97]. Treatment with sibutramine is associated with the reduction in circulating triglyceride levels and a rise in HDL-C, the latter of which might be partially independent from the effects of weight loss [98]. However, it is not yet known whether antiobesity drug treatments reduce cardiovascular morbidity and mortality. The ongoing Sibutramine Cardiovascular Outcomes Trial (SCOUT) and Comprehensive Rimonabant Evaluation Study of Cardiovascular Endpoints and Outcomes (CRESCENDO) trials will assess the effects of sibutramine and

rimonabant, respectively, on cardiovascular events and mortality (ClinicalTrials.gov identifiers NCT00234832 and NCT00263042).

Glycemic control

The RIO-Diabetes trial enrolled 1045 overweight and obese subjects with type 2 diabetes. Subjects in this trial had to have taken metformin or sulfonylurea monotherapy for at least six months, and to have fasting plasma glucose levels between 100 and 271 mg/dL, and hemoglobin A_{1c} (HbA_{1c}) levels between 6.5% and 10%. All subjects continued treatment with metformin or sulfonylurea throughout the study. Subjects treated with rimonabant (20 mg/d for one year, intention-to-treat population) had significant decreases in body weight and improvements in glycemic control compared with placebo [12]. Moreover, rimonabant might have additive effects to standard, background oral antidiabetic therapy, because the favorable change in mean HbA_{1c} was observed in subjects receiving metformin or sulfonylureas. Also, this effect of rimonabant appeared to be partly independent of weight loss. The safety and effectiveness of combining drugs with different mechanisms of action for the treatment of obesity and diabetes is under investigation in the REASSURE and ALLEGRO trials. In these trials, obese patients with type 2 diabetes receive concomitant treatment with rimonabant, metformin and sulfonylureas. REASSURE is a trial of rimonabant in subjects not adequately controlled on metformin and sulfonylureas. In the ALLEGRO trial, glimepiride treatment is compared with rimonabant plus metformin combination treatment. (ClinicalTrials.gov identifiers NCT00546325 and NCT00449605).

Safety data with CB1 receptor blockade

The overall withdrawal rate in the RIO studies was high [9–12] and was similar to the overall withdrawal rate observed in other obesity trials [99]. The rate of discontinuation due to adverse events in subjects treated with rimonabant 20 mg/d was 15.0%, 14.5%, 12.8% and 15.0% compared with 7.0%, 9.2%, 7.2% and 5.5% in the placebo group in RIO-Lipids, RIO-Europe, RIO-North America and RIO-Diabetes, respectively [100]. Adverse events leading to study discontinuation over one year of treatment in subjects treated with rimonabant 20 mg/d included depressive disorders, nausea, anxiety and dizziness. With regard to nausea, both preclinical and clinical studies support a role of the ECS in nausea [101,102]. In a rat model of anticipatory nausea, a gaping reaction is induced during exposure to a context previously paired with lithium chloride-induced illness [102]. Pretreatment with THC suppressed the lithium-induced gaping reaction [102] and administration of SR141716 potentiated the lithium-induced nausea [103,104]. Furthermore, previous studies carried out in ferrets [105] and musk shrews [106] had also shown that CB_1 receptor agonists and antagonists, respectively, inhibit and worsen vomiting induced by various agents.

Adverse events associated with rimonabant 20 mg/d in the RIO trials included very minor neurological symptoms, such as sensory changes (e.g. dizziness), motor impairment (e.g. tremor) and cognitive disorders (e.g. amnesia) [107]. The relative risk for all neurological adverse events was 2.5, 1.0, 1.3 and 2.9 in RIO-Lipids, RIO-Europe, RIO-North America and RIO-Diabetes, respectively. Psychiatric adverse events were, instead, more of a concern. In fact, rimonabant 20 mg/d was associated with about a twofold increase in the risk of anxiety and depressed mood [107]. Persons with a history of severe depression or other psychiatric disorders, or who had prior use of antidepressant medications, were excluded from all the RIO studies. In 2007, the sponsor withdrew the New Drug Application before the FDA gave a decision. Issues of note were related to drug safety, particularly those in the psychiatric domain in subjects with depression [108]. Thus, further clarification of patient populations at risk of developing psychiatric adverse events and a comprehensive program to mitigate these risks are needed. Of note, both high and low ECS activity might be linked with mood disorders [109,110] and different doses of CB_1 receptor antagonists can either worsen or ameliorate signs of mood disorders in laboratory animals in a CB_1-receptor-dependent manner. Rimonabant was approved by the European Medicines Agency (EMEA) in June 2006 [111]; however, the EMEA recently recommended that rimonabant is contraindicated in patients with ongoing major depression and in patients being treated with antidepressants [111,112].

The percentage of subjects who withdrew from the 12-week taranabant dose range-finding study was approximately 33% (175/533) [59]. The percentage of subjects who withdrew due to adverse events was 10.5% ($n = 11$) in the placebo group compared with 4.7% ($n = 5$), 4.6% ($n = 5$), 16.2% ($n = 17$) and 10.2% ($n = 11$) for taranabants 0.5, 2, 4 and 6 mg/d, respectively [59]. There was a dose-related increased incidence of clinical adverse events with taranabant treatment, including mild to moderate GI and psychiatric effects [59]. Persons with a history of significant depression, or other psychiatric disorders, were excluded from the taranabant dose range-finding study [59]. The one-year result of the study with taranabant was recently communicated at the meeting of the European Association of Atherosclerosis. This study showed that at all doses efficacious at reducing body weight, taranabant exerted psychiatric adverse events to an extent very similar to that observed in the RIO trials [113,114].

Conclusions

The expression of the ECS in both brain and peripheral organs indicates that CB_1 receptor antagonism might provide a rational basis for targeting multiple tissues involved in energy homeostasis through pharmacological modulation of a single receptor. In human trials, the CB_1 receptor antagonist rimonabant not only induced weight loss, but also improved effects on serum lipid levels and glycemic control. Animal and cell culture studies indicate that CB_1 receptor antagonism has direct effects on lipid metabolism in adipose tissue and liver, and possibly glucose metabolism in adipose tissue and skeletal muscle. In clinical trials, rimonabant appeared to have a weight-loss-independent effect on increasing plasma levels of adiponectin, an adipocyte-derived hormone that has an important regulatory role in lipid and glucose metabolism. Thus, increased adiponectin levels might account for some of the metabolic effects of CB_1 receptor antagonism. Further preclinical and clinical studies with CB_1 receptor antagonists are needed to define and understand fully the relative weight of central versus peripheral effects on the beneficial actions of these compounds on metabolic disorders, and to determine the plausible efficacy of yet-to-be-developed peripherally restricted CB_1 receptor antagonism in humans.

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