Dysregulation of the endocannabinoid system (ECS) is a universal and, perhaps, causative feature of obesity. Central nervous system (CNS) circuits that regulate food intake were initially believed to be the targets for dysregulation. However, it is increasingly evident that endocannabinoids affect food intake, energy expenditure and substrate metabolism by acting on peripheral sites. Cannabinoid type 1 receptor (CB1r) antagonists can effectively treat obesity and associated metabolic alterations but, unfortunately, cause and exacerbate mood disorders. Drugs restricted to act on peripheral CB1rs might be safer and more effective, retaining the anti-obesity effects but lacking the adverse neurodepressive reactions. This review summarizes the emerging roles of the ECS in energy balance and discusses future pharmacological approaches for developing peripherally restricted CB1r antagonists.

Endocannabinoid activity and energy metabolism

Endocannabinoids (ECs) are arachidonic acid derivatives that act as endogenous ligands for two main receptors: cannabinoid receptor type 1 (CB1r) and type 2 (CB2r). The best studied ECs are N-arachidonoyl ethanolamine, also known as anandamide (AEA), and 2-arachidonoyl glycerol (2-AG). The ECs, their receptors, and the enzymes responsible for their synthesis and degradation constitute the endocannabinoid system (ECS) [1]. Unlike canonical neurotransmitters, ECs such as 2-AG are not produced and stored in vesicles, but are synthesized on demand from lipids of the post-synaptic membrane. It is believed that they diffuse and act on CB1rs located in the presynaptic membrane, modulating excitatory and inhibitory neurotransmission in the central (CNS) and peripheral (PNS) nervous systems. In addition to their general function as synaptic neuromodulators, ECs are implicated in the regulation of energy metabolism. Because the ECS is dysregulated in obesity [2], pharmacological restoration of normal ECS activity represents a novel approach to fighting obesity and its metabolic complications.

The well-known hunger-inducing effects of exogenous cannabinoids and the prominent expression of CB1r in the CNS [3] focused early studies on CNS areas where the receptor is known to exert orexigenic actions (see Glossary). Indeed, the first selective CB1r antagonist (SR141716, or rimonabant) caused anorexia and weight loss in rodents [4,5]. It was soon recognized that weight loss via CB1r blockade is mediated not only by anorectic mechanisms, but also by metabolic actions on peripheral organs.

Clinical trials confirmed that rimonabant effectively induces weight loss and improves lipid and glucose metabolism in obese humans [6]. Unfortunately, the trials also revealed adverse psychiatric side effects, which led to the withdrawal of CB1r antagonists as anti-obesity agents for clinical use.

Recent evidence indicates that peripheral CB1rs modulate satiety and energy expenditure [7–9]. Thus, CB1r antagonists that do not cross the blood–brain barrier (BBB) would provide a novel pharmacological approach to controlling obesity without the psychiatric side effects observed with global CB1r antagonists. In this review, we discuss recent progress in understanding the ECS role in...
obesity pathogenesis, in particular the potential mechanisms by which ECs contribute to energy balance modulation at both central and peripheral sites.

**Central ECs and the control of food intake**

EC signaling in the brain stimulates orexigenic pathways by acting in hypothalamic areas that control food intake [3]. In addition to the homeostatic modulation of feeding behavior, ECs can influence neural pathways by affecting the motivational and pleasurable aspects of eating [non-homeostatic feeding behavior] [2]. A detailed description of the mechanisms behind EC-mediated control of feeding behavior is summarized in Box 1. Notably, ECs might also control food intake by acting in the area postrema (AP) of the brain stem, a cerebral structure located outside the BBB that might represent a target for the anorectic effects of peripherally restricted compounds [10]. This potential role for CB1r in the AP was recently suggested in a preclinical study to test the anti-obesity efficacy of a new peripherally restricted CB1r antagonist [9]. However, the role of the AP as a potential target for the anorectic effects of peripherally acting CNS antagonists awaits confirmation by testing, for example, the efficacy of these drugs in obese animals with a specific CB1r ablation in this area.

In conclusion, the anti-obesity action of CB1r antagonism is ascribed to ECS modulation of synaptic activity in CNS centers controlling feeding behavior via homeostatic and non-homeostatic mechanisms. It is now established, however, that EC action in the brain not only affects feeding behavior but also influences descending peripheral neuronal pathways involved in the control of energy balance, as described below.

**ECs beyond food intake: energy expenditure and thermogenesis modulation**

A large body of evidence suggests that the attenuation of diet-induced obesity (DIO) observed with CB1r blockade is due not only to anorectic effects, but also to a marked increase in energy expenditure [4,11–14]. One of the most striking effects of CB1r antagonism is the marked activation of brown adipose tissue (BAT) function [15]. Unlike white adipose tissue (WAT), BAT can dissipate calories into heat by uncoupling oxygen consumption from ATP synthesis (non-shivering thermogenesis) [16]. This unique function of BAT is essential for homeostatic control of body temperature, as well as body weight. Indeed, evidence in rodents indicates that stimulation of BAT functional activity might be an effective strategy for preventing or treating obesity [16].

Neuroanatomical evidence suggests that CB1r in the CNS can stimulate BAT thermogenesis, thereby promoting weight loss through feeding-independent mechanisms. Melanocortin receptor type 4 (MC4r)-expressing neurons in the PVN regulate BAT activity [17,18] and CB1r antagonists are abundantly expressed in the PVN, and probably modulate outputs from the PVN to BAT [19]. In addition, MC4r activity is modulated by ECs [20]. ECs may also modulate BAT activity via MCH neurons in the LH, whose projections to the caudal brainstem and spinal cord innervate BAT [20].

Functional data also support the notion that CB1r regulates BAT metabolic function; pharmacological CB1r blockade in rodents increases mRNA levels of UC1P1 (a BAT-specific marker and mediator of thermogenesis) [12,21] and the expression of genes regulating BAT mitochondrial and thermogenic activity [22]. Conversely, UC1P1 expression is decreased in cultured brown adipocytes treated with a non-selective CB1r/CB2r agonist [23].

Direct evidence that CB1r blockade stimulates BAT in vivo comes from a study showing that treatment with the drug rimonabant in cold-exposed mice increases BAT activity [7]. The increased BAT function induced by CB1r antagonism seems to be mediated by sympathetic innervation because BAT denervation (loss of nerve supply) abolishes the increased thermogenic activity induced by rimonabant [7,15]. The sympathetic nervous system (SNS)-dependent stimulation of BAT induced by CB1r blockade might arise from a stimulatory effect on mitochondrial biogenesis. Indeed, BAT isolated from mice with a CB1r deletion in neuronal tissues exhibits increased mitochondrial density and this phenotype is reversed by SNS denervation [7].

Increased BAT mitochondrial activity may have an impact on the whole-body metabolic changes observed after treatment with CB1r global antagonists. The great ability of BAT mitochondria to oxidize fatty acids [24] might cause increased energy expenditure and loss of fat.
mass. Indeed, SNS-induced stimulation of BAT activity by rimonabant can contribute to the body-weight-reducing effects of the drug [15]. The SNS-dependent action of CB1r antagonists on BAT function is not an exclusive mechanism. An alternative mechanism has recently been postulated, whereby rimonabant causes in vitro transdifferentiation of white adipocytes to brown adipocytes [25].

Although the role of the ECS in BAT functioning in rodents is now well established, the existence and nature of this interaction in humans remain unknown. Contrary to previous belief, adult humans retain active BAT deposits that might be exploited as pharmacological targets to tackle obesity [26], but the role of BAT in human obesity has still to be clarified. For example, it is not clear whether, as seen for mice, BAT activation in humans can influence energy expenditure at the whole-body level [27].

Although BAT activation can be measured easily and non-invasively via positron emission tomography (PET) imaging, the intriguing possibility that CB1r antagonists might stimulate BAT function in humans cannot be tested owing to the withdrawal of these drugs from clinical use. Because the amount of functionally activatable BAT varies among individuals [27] and CB1r antagonists promptly activate BAT function after the first dose, analysis of BAT function via PET imaging might represent an excellent early predictive test for the identification of patients who respond to these classes of drugs.

**Contribution of peripheral organs expressing CB1r in energy balance**

In recent years, the ECS was implicated in the modulation of energy metabolism in peripheral tissues such as WAT, skeletal muscle, endocrine pancreas, liver and small intestine [28]. WAT is the most-studied peripheral tissue in this context, as nicely summarized in [29].

In brief, ECs have several effects on adipocytes, such as promotion of adipogenesis via PPAR-γ, triglyceride synthesis and glucose uptake [30]. Conversely, CB1r blockade induces lipolysis in adipocytes, an effect that may cause the whole-body stimulation of energy expenditure observed with CB1r antagonists. Indeed, rimonabant treatment in rats induces WAT lipolysis, thereby increasing the availability of free fatty acids (FFAs) for oxidation and possibly increasing energy expenditure [31]. Although increased WAT lipolysis might represent the first cause of the beneficial effect of CB1r antagonists on energy expenditure, the molecular mechanisms remain unclear.

Recent evidence supports the idea that CB1r modulation of the SNS might control lipolysis and energy expenditure. Mice with a selective CB1r deletion in the principal forebrain and sympathetic neurons display resistance to DIO, fat accumulation and metabolic dysfunction. Interestingly, their phenotype was indistinguishable from that of mice with a global CB1r deletion, which highlights the importance of CB1r in these neurons in energy balance control [7]. In agreement with previous results [31], reduced neuronal CB1r signaling stimulated lipid oxidation, reduced metabolized energy and increased BAT thermogenesis. Elevated sympathetic activity is required for stimulation of BAT function, but it is plausible that increased SNS activity also underlies the whole-body increase in lipid oxidation in these mice. Increased norepinephrine release resulting from neuronal CB1r blockade might in fact stimulate lipolysis in WAT and increase lipid oxidation due to increased FFA availability [7]. This hypothesis is supported by a recent study showing that the lipolytic property of rimonabant cannot be directly attributed to its action on adipocyte receptors, but rather to the activation of circuits connecting the SNS and the adrenal gland [32]. The mechanism by which neuronal CB1r controls energy balance is illustrated in Figure 1.

Although the ultimate targets of EC-mediated control of energy balance in adipose tissue could be the CB1rs located at WAT nerve endings, the CB1rs expressed in adipocytes might contribute to the control of WAT metabolism. CB1rs expressed in adipocytes control mitochondrial biogenesis. Indeed, CB1r antagonists exhibit increased mitochondrial activity [21] and expression of genes for mitochondrial biogenesis in WAT [22,23]. Accordingly, CB1r agonist treatment decreases mitochondrial biogenesis in mouse and human cultured white adipose cells [33].

Multiple lines of evidence support the hypothesis that adipocytes not only function as energy storage, but can also flexibly and significantly increase their oxidative properties by changing their mitochondrial density [24]. Thus, CB1r expressed on adipocytes might directly influence whole-body energy metabolism by modulating mitochondrial biogenesis and oxidative activity in adipose tissue.

The ability of CB1r blockade to increase whole-body oxidative capacity might also be secondary to induction of the hormone adiponectin, whose actions in stimulating energy expenditure and improving glucose homeostasis are well established [34]. The ability of CB1r modulation to influence adiponectin production, however, is still controversial and requires further investigation [35–42].

CB1r located in skeletal muscle might represent another important mediator of the increased lipid oxidation and energy expenditure observed with CB1r antagonists. In vitro evidence demonstrates that CB1r blockade increases oxygen consumption in skeletal muscle of ob/ob mice [43]; however, it remains to be verified whether this effect contributes to the whole-body increased energy expenditure induced by rimonabant.

CB1r in the liver might help in part to regulate fatty acid oxidation [44]. Intrahepatic EC levels might contribute to fat accumulation in the liver by activating hepatic CB1r, suppressing hepatic carnitine-palmitoyltransferase 1 activity and decreasing fat oxidation, leading to a decrease in energy expenditure [44]. In conclusion, these data indicate that the action of ECs in peripheral organs such as adipose tissue, skeletal muscle and liver can influence energy expenditure and substrate metabolism.

The CB1rs expressed in these tissues, as well as in the nerve endings modulating peripheral metabolic function, have important roles. In this regard, it is important to note that ECS activity in peripheral tissues is under the control of hormones that regulate energy balance and act in the brain, such as leptin and insulin [45]. This suggests that hormones such as leptin might control energy metabolism in peripheral tissues in part by modulating ECS activity via efferent neural pathways.
Peripheral CB1r and metabolic dysregulation associated with obesity

In clinical studies using CB1r antagonists to treat obesity, statistical modeling revealed that plasma glucose and lipid levels were decreased to an extent greater than that predicted by the amount of body-weight loss [38], which suggests a direct role for CB1r antagonism in modulating lipid and glucose homeostasis, independent of its weight-reducing effects. However, there are no known studies in humans specifically designed to address this question or to confirm the statistical observation [41].

Preclinical data suggest that CB1r blockade might improve lipid and glucose metabolism during obesity. CB1r in skeletal muscle is a potential target by which the ECS can control glucose metabolism and insulin sensitivity. CB1r antagonism increases glucose uptake in L6 myotube cultures [46], primary cultures of human skeletal muscle cells [47] and soleus muscle isolated from obese animals [43,48]. Moreover, pharmacological inhibition or activation of CB1r in cultured myotubes can reinforce or dampen intracellular insulin signaling, respectively [49]. However, such evidence does not directly implicate CB1rs located in skeletal muscle in the regulation of insulin sensitivity during obesity.

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In view of the potent effect of rimonabant in stimulating glucose uptake in BAT [7], an intriguing hypothesis is that the ECS in BAT might have a role in ameliorating the insulin sensitivity induced by CB1r antagonism, because BAT has the highest insulin-dependent glucose uptake per unit of weight.

The endocrine pancreas also responds to manipulation of ECS activity; however, the role of the ECS in insulin secretion is unclear owing to contradictory data indicating that CB1r activation is coupled to both increased and decreased insulin secretion [28,50].

A prominent role for the hepatic ECS has been highlighted using conditional knockout mice with a liver-specific CB1r deletion [51]. Deletion of CB1r in the liver decreases hepatic lipogenesis and ameliorates hypercholesterolemia, hypertriglyceridemia and hepatocellular damage induced by a high-fat diet [51]. Moreover, overactive ECS signaling might induce an increase in plasma triglyceride levels associated with reduced plasma triglyceride clearance [52]. In conclusion, both clinical and preclinical data indicate that CB1r antagonism improves the metabolic profile in obese individuals. CB1rs located in peripheral sites seem to be involved in these effects, but it is still not clear, especially in humans, whether EC action at these sites affects the metabolic profile directly or indirectly via adiposity reduction.

ECS overactivity associated with obesity

Obesity is characterized by upregulation of CB1r and increased levels of EC, both in circulation and in tissues. The first evidence of increased EC levels was provided by a study that revealed pathologically elevated ECs in the hypothalamus of obese animals, possibly because of leptin deficiency or resistance; leptin is a potent inhibitor of the CNS and peripheral EC levels under physiological conditions [5].

Moreover, it is clear from recent studies in both humans and animals that obesity also overactivates the ECS in peripheral organs such as WAT [36,39], BAT [7], liver [44], pancreas [36] and skeletal muscle [28]. Interestingly, obese humans have increased EC levels in visceral adipose tissue but decreased levels in subcutaneous depots [29], which...
suggests that the imbalance between ECS tone in visceral and subcutaneous fat can lead to greater accumulation of intra-abdominal compared with subcutaneous fat. Preferential activation of ECS tone in visceral fat has been proposed as a causative mechanism for insulin resistance and obesity-associated atherosclerosis [29].

Whether ECS overactivation is the causal link for the metabolic dysregulation associated with obesity is the subject of intense scrutiny. Obesity is commonly characterized by both leptin and insulin resistance either in the CNS or in peripheral tissues. Leptin can reduce EC tone in the hypothalamus of lean individuals [5] and insulin affects EC enzymatic machinery in both adipocytes and human subcutaneous depots [30]. Thus, it is possible that insulin and leptin resistance can cause and/or worsen the elevation of ECS tone in obesity. Leptin can regulate ECS tone in adipose tissue via a neural signal. Indeed, intrahypothalamic delivery of leptin suppresses AEA production in WAT, inhibits peripheral lipogenesis and stimulates lipolysis [45]. This suggests that the lipolytic effects of CNS leptin action might be mediated by its ability to inhibit WAT ECS tone, and that the central leptin resistance in obesity might be responsible for ECS over-activation [45]. In turn, loss of the anti-lipogenic and lipolytic effects of CNS leptin in obesity could be explained by its failure to inhibit lipogenic ECS tone.

To date, most of our knowledge on the role of ECS overactivity is based on measurements of ECS components in plasma, tissues or biopsies [28]. Although it seems premature to consider circulating ECs as markers of obesity, in particular because we do not know the source of ECs measured in the blood, a series of studies has highlighted the strong positive association between circulating levels of 2-AG and AEA and obesity (Table 1) [36,53–59]. However, these data should be interpreted with caution because (i) the studies were performed on small cohorts of patients who were not always age- and gender-matched and (ii) sample collection and techniques used to measure EC levels were not standardized [60], which results in great variability in EC levels obtained from different laboratories. These problems prevent current use of EC plasma or tissue levels as biomarkers for diagnostic and therapeutic goals. Standardized assays and the establishment of reproducible reference intervals in lean and obese humans are urgently needed.

Is it really the end of the line for CB1r as an anti-obesity target? Lessons from recently discovered peripherally restricted CB1r antagonists
As discussed above and summarized in Box 2, health authorities did not approve rimonabant for clinical use because of its adverse side effects. New peripherally restricted CB1r antagonists are likely to be devoid of psychiatric side effects and might represent a more effective and safer strategy for counteracting obesity [61]. Lead compounds in this class of CB1r antagonists have been designed and tested in rodents during the last few years.

The first peripheral CB1r/CB2r antagonist to be synthesized and tested was URB447, which promotes body-weight loss to an extent that is similar to that of rimonabant in Ob/Ob mice [62]. However, whether this drug fails to completely penetrate the CNS and the mechanism of its exclusion are still the subject of debate because of limited experimental data in support of its peripheral selectivity. This crucial issue also remains to be settled for other recently synthesized peripherally restricted CB1r antagonists and neutral antagonists [63,64].

The compound AM6545 has very promising properties and more convincing evidence of its peripheral selectivity has been obtained [8]. Compared to rimonabant, AM6545 shows a similar affinity for CB1r and similar behavioral effects (catelepsy, hypomotility and hypothermia), but AM6545 has markedly decreased brain penetration owing to reduced lipid solubility and a high affinity for the ABC transporter P-glycoprotein that mediates its eflux from the CNS [8]. In DIO mice, AM6545 induces body-weight and adiposity reductions, but at a lower magnitude than observed when treating animals with the global CB1r antagonist rimonabant. However, as a peripherally restricted drug, AM6545 retains a marked ability to improve glucose tolerance, increase adiponectin levels, reduce leptin and insulin levels, and decrease hepatic triglycerides [8]. These results suggest that, despite lower potency compared to global CB1r antagonism, peripheral CB1r targeting could be an effective strategy for sustained and clinically significant effects against obesity and its metabolic complications.

Intriguingly, a small and transient reduction in food intake was observed with AM6545 administration. However, this was insufficient to induce the beneficial metabolic effects of the drug, as demonstrated by pair-feeding [8]. This transient reduction in food intake was also observed in wild-type and CB1r total knockout mice and in
<table>
<thead>
<tr>
<th>Subjects</th>
<th>BMI (kg/m²) and waist (cm)</th>
<th>Results</th>
<th>Plasma concentrations of ECs</th>
<th>Correlation</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 postmenopausal lean (F) vs 20 postmenopausal obese (F)</td>
<td>BMI and waist 23.5±0.4 and 76±1 (lean) 38.3±0.7 and 109±2 (obese)</td>
<td>AEA in obese vs lean</td>
<td>AEA: 2 pmol/ml lean* AEA: 2.5 pmol/ml obese* 1/2 AG: 16 pmol/ml lean* 1/2 AG: 23 pmol/ml obese*</td>
<td>• AEA positively correlates with BMI • 1/2AG levels positively correlate with waist</td>
<td>[53]</td>
</tr>
<tr>
<td>8 healthy (5M/3F) vs 10 (4M/6F) diabetic obese</td>
<td>BMI: 28.6±1.9 (healthy) 33.5±3.0 (diabetic obese)</td>
<td>AEA in diabetic obese vs healthy</td>
<td>AEA: 1.8 pmol/ml healthy* AEA: 2.9 pmol/ml diabetic obese * 2-AG: 0.8 pmol/ml healthy * 2 AG: 2.9 pmol/ml diabetic obese *</td>
<td>n.d.</td>
<td>[36]</td>
</tr>
<tr>
<td>20 lean (10M/10F) vs 20 subcutaneous obese (10M/10F) vs 20 visceral obese (10M/10F)</td>
<td>BMI and waist: 25±2 and 87±1.3 (M lean) 24±1 and 69.5±3 (F lean) 38±7 and 140±5.6 (subcutaneous obese M) 34±8 and 132±4.9 (subcutaneous obese F) 35±7 and 138±5.4 (visceral obese M) 34±7 and 129±4.5 (visceral obese F) = AEA obese vs lean</td>
<td>AEA in obese vs healthy</td>
<td>AEA: 2.1 pmol/ml in all female* AEA: 1.9 pmol/ml in all male* 2-AG: 5.3 pmol/ml lean* 2-AG: 6.1 pmol/ml obese subcutaneous* 2-AG: 9.3 pmol/ml obese visceral*</td>
<td>• 2-AG positively correlates with BMI, percent body fat and visceral fat area in both sexes</td>
<td>[54]</td>
</tr>
<tr>
<td>62 (M)</td>
<td>BMI and waist: 27.4±4.5 and 94.6±12.3</td>
<td>n.d.</td>
<td>AEA: 2.86±0.8 pmol/ml 2-AG: 1.02±0.5 pmol/ml</td>
<td>• AEA negatively correlates with intra-abdominal adipose tissue • 2-AG positively correlates with BMI, waist, triglycerides, fasting insulin levels • 2-AG negatively correlates with HDL cholesterol and adiponectin</td>
<td>[55]</td>
</tr>
<tr>
<td>20 (6F/4M) lean normoinsulinemic vs 10 (6F/4M) obese hyperinsulinemic</td>
<td>BMI: 21.9 (normal); BMI: 35.8 (obese)</td>
<td>AEA in obese hyperinsulinemic</td>
<td>AEA: 4.7 pmol/ml lean* AEA: 15.7 pmol/ml obese* 2-AG: 3.1 pmol/ml lean* 2-AG: 3.3 pmol/ml obese*</td>
<td>• AEA positively correlates with BMI, waist, visceral tissue, subcutaneous tissue and triglycerides • 2-AG positively correlates with triglycerides</td>
<td>[56]</td>
</tr>
<tr>
<td>7 M non diabetic vs 12 M diabetic</td>
<td>BMI: 26.3 (non diabetic); BMI: 28.5 (diabetic) = AEA and = 2-AG between the 2 groups</td>
<td>AEA: 5.2 pmol/ml non-diabetic* AEA: 5.6 pmol/ml diabetic* 2-AG: 1.5 pmol/ml non-diabetic 2-AG: 1.6 pmol/ml diabetic*</td>
<td>• No correlation of AEA with any metabolic parameters • 2-AG positively correlates with triglycerides</td>
<td>[56]</td>
<td></td>
</tr>
<tr>
<td>49 M before and after year lifestyle modifications</td>
<td>BMI: 30.9±3.3 (before) BMI: 28.8±3.6 (after)</td>
<td>AEA (-7.1%) after lifestyle modification</td>
<td>AEA: 5.2 pmol/ml before intervention* 2-AG: 1.58 pmol/ml before intervention*</td>
<td>• Decrease in 2-AG but not AEA levels positively correlates with reduction in visceral adipose tissue</td>
<td>[57]</td>
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<tr>
<td>48 (17M/31F) normal weight vs 96 (43M/53F) severely obese</td>
<td>BMI 24.7±1.2 normal weight Waist 103.3±0.4 normal weight males Waist 93.7±3.3 normal weight females BMI 49.2±3.2 severely obese Waist 151.2±8.1 severely obese males Waist: 140.7±7 severely obese females</td>
<td>Trend to ↑ AEA in severely obese</td>
<td>Mean AEA 12.8 (11.4 14.5) pmol/ml normal weight Mean AEA 14.5 (13.6 15.6) pmol/ml severely obese Mean 2-AG 5.6 (4.6 6.8) pmol/ml normal weight Mean 2-AG 5.4 (4.8 6.1) pmol/ml severely obese. 95% CI for all the data</td>
<td>• AEA positively correlates with FAAH 386 A mutant (enzyme degrading AEA)</td>
<td>[58]</td>
</tr>
<tr>
<td>27 (M)</td>
<td>Caucasians: BMI 32.8; waist 103.2 American Indians: BMI 33.2; waist 105.9 African Americans: BMI 37.8; waist 115.3</td>
<td>↑AEA in obese</td>
<td>AEA: 1.1±0.5 pmol/ml 2-AG: 37.6±30.7 pmol/ml</td>
<td>• AEA positively correlates with waist, BMI, fasting insulin, insulin area under OGTT • No association of AEA and 2-AG with leptin and measures of energy expenditure</td>
<td>[59]</td>
</tr>
</tbody>
</table>

M, male; F, female; BMI, body mass index; n.d., not determined; * values derived from figures; ↑, increase; ↑↑, marked increase; =, no change; ↓, decrease; CI, confidence interval; OGTT, oral glucose tolerance test.
DIO rats [65], but not in CB1r/CB2r knockout mice treated with AM6545 [9]. These observations suggest that the anorectic effects of AM6545 might be mediated via CB2r rather than CB1r antagonism, a fact supported by the Ki value for AM6545 against CB2r (K_i=523 nM). CB2r agonism has been shown to inhibit food intake in C57BL/6 mice [66]; in agreement with this observation, CB2r knockout mice are hyperphagic compared to wild-type mice [67]. However, for unknown reasons, pharmacological modulation of CB2r activity does not influence food intake in some genetic strains of mice [66]. Therefore, the role of CB2rs in the physiologic control of feeding behavior must still be identified.

Another interesting potential mechanism by which AM6545 reduces food intake is via antagonism of CB1rs located in the gut. The importance of CB1r in the gastrointestinal (GI) tract in the regulation of feeding behavior is suggested by observations that AEA and 2-AG levels in the small intestine of rats are regulated by fasting/refeeding [68,69] and that EC-mediated hyperphagia requires intact capsaicin-sensitive afferent nerve fibers [68]. Another study suggests that the orexigenic action of intestinal ECs occurs via stimulation of CB1r located in vagal afferent neurons, whose activity is modulated by the hormone cholecystokinin [70].

By contrast, subdiaphragmatic truncal vagotomy or complete surgical resection of vagal afferent fibers does not affect the ability of rimonabant to induce anorexia [71]. Like rimonabant, AM6545 still suppressed food intake in rats with a complete subdiaphragmatic truncal vagotomy, which implies that an intact vagus nerve is not required in the mediation of this effect [9]. Therefore, although strong evidence exists to support the notion that CB1r antagonists might induce anorexia via gut receptors, the anatomical site for these ‘peripheral anorectic effects’ is still elusive. Alternatively, the anorectic effects of both rimonabant and the peripherally restricted AM6545 could be mediated by an interaction with CB1r in the AP [9].

In conclusion, preclinical screening of new peripherally restricted CB1r antagonists has revealed that CB1rs expressed in peripheral tissues are effective targets for control of body weight and metabolic dysregulation in obesity. Moreover, the ability of these drugs to modify food intake highlights the emerging possibility that CB1rs expressed in the GI tract can affect feeding behavior.

Concluding remarks

As shown in Box 3, important questions regarding the mode of action of the ECS in regulating energy and fuel metabolism at central and peripheral sites await conclusive answers. However, increasing evidence strongly supports the notion that the ECS modulates energy metabolism at multiple sites, including the CNS, the PNS and peripheral non-neuronal tissues. These peripheral effects provide a conceptual basis for the anti-obesity properties of peripherally active drugs. Indeed, the effectiveness of new antagonists with a restricted action on peripheral CB1rs in preclinical studies gives new hopes for their future clinical use as anti-obesity drugs, once a lack of CNS adverse effects is confirmed in clinical trials.

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**Box 3. Outstanding questions**

- What is the primary mechanism by which CB1r antagonism promotes weight loss?
- What is the relative contribution of neuronal or extraneuronal CB1r in the regulation of energy metabolism?
- What is the pathophysiologic role of the ECS in the pancreas or skeletal muscle? Does the ECS in these tissues regulate glucose and lipid metabolism?
- Is human BAT involved in the anti-obesity properties of CB1r antagonists?
- Are the peripherally restricted CB1r antagonists as effective in humans as in animals at improving obesity and associated comorbidities?
- Which patients are more likely to respond to CB1r antagonists, and can this propensity be predicted with specific and sensitive biomarkers?

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