Endocannabinoids in obesity: brewing up the perfect metabolic storm?

Christopher Lipina¹, Wiebke Rastedt¹, Andrew J. Irving¹ and Harinder S. Hundal¹*

In recent years, abnormal regulation of the endocannabinoid system (ECS) has been proposed as a key factor in the development of obesity-related metabolic disorders such as diabetes. Indeed, this signaling system which includes the cannabinoid type 1 and 2 receptors (CB1R and CB2R) and their endogenous lipid ligands, has been shown to influence feeding behavior, energy expenditure, as well as glucose and lipid metabolism. Importantly, blocking CB1R function has been demonstrated to counteract metabolic aberrations associated with obesity in various murine models and in humans. Here we provide an update on recent findings describing the role of the ECS in energy balance and metabolism, and explore how recent experimental and clinical studies have delivered new insights into the therapeutic potential of this physiological system as a means of treating obesity-induced metabolic disorders.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by both hyperinsulinemia and hyperglycemia, and can develop as a result of genetic predisposition and/or involve other risk factors, in particular diet-induced obesity. Two key pathological features of this metabolic disease include insulin resistance and impaired insulin secretion from pancreatic β-cells. Indeed, the resulting abnormalities in systemic energy homeostasis are often associated with increased food intake, as well as dysregulated metabolism in the form of altered uptake and storage of nutrients in peripheral tissues, such as liver, adipose, and skeletal muscle.

Although originally identified as being important in neuromodulation, the endocannabinoid system (ECS) has subsequently been implicated in a wide range of physiological and pathological processes including cardiovascular function, cancer, inflammation, and immunity.¹⁻⁶ Importantly, there is growing evidence that this signaling system plays an integral role in energy homeostasis, consistent with its observed dysregulation in the obese state.⁷⁻¹⁰ This article provides an overview of current research in the involvement of the ECS in energy balance and metabolism, and discusses possible future directions in developing novel treatments for obesity-related disorders such as T2DM.

The ECS: Key Components and Modulatory Tools

Integral to a functional ECS are cannabinoid receptors whose activity is modulated by endogenous arachidonic acid-derived lipids called endocannabinoids. The main endocannabinoids identified to date are anandamide (arachidonylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG).¹¹,¹² Both these lipids are thought to mediate their cannabimimetic properties through the classical G-protein coupled cannabinoid receptors: cannabinoid receptor type 1 (CB1R) and type 2 (CB2R).¹¹,¹² However, whereas anandamide displays greater affinity toward CB1R and only weakly activates CB2R, 2-AG can potently activate both receptors.¹³⁻¹⁶ Furthermore, there is emerging evidence that this signaling system plays an integral role in energy homeostasis, consistent with its observed dysregulation in the obese state.⁷⁻¹⁰
evidence that endocannabinoids may also mediate some of their biological effects through alternative molecular targets, such as the G-protein coupled receptors GPR55 and GPR18, or by regulating peroxisome proliferator-activated receptors (PPARs).17-19

Endocannabinoids are synthesized at the plasma membrane in response to various acute stimuli.20 Following research focussed on manipulating the levels of these important bioactive lipids, a number of enzymes implicated in the synthesis of anandamide and 2-AG have now been identified. Anandamide is formed as a result of phospholipase D-mediated hydrolysis of its phospholipid precursor N-arachidonyl phosphatidylethanolamine (NAPE). In contrast, 2-AG is generated following sequential hydrolysis of membrane inositol phospholipids by phospholipase C and arachidonate-containing diacylglycerols by diacylglycerol lipase (see Figure 1). In addition to these synthetic pathways, enzymes that catalyze the degradation of anandamide and 2-AG have also been characterized, including fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (see Figure 1).21 Furthermore, alternative enzymes such as lysosomal N-acylethanolamine hydrolyzing acid amidase (NAAA) and cyclooxygenase 2 (COX2) are known to participate in the metabolism of endocannabinoids22-24 (see Figure 1).

In addition to regulating the levels of endocannabinoids themselves, modulating ECS function can also be achieved through altering cannabinoid receptor activity. Indeed, the application of mixed or selective cannabinoid receptor ligands is a common strategy deployed to decipher the role of the ECS in energy homeostasis (see Table 1).8,25-28 Importantly, it should be noted that inhibitory compounds can act either by competitively binding and blocking activation of a receptor by an agonist (i.e., as an antagonist), and/or alternatively as inverse agonists through inhibiting constitutive receptor signaling. For example, rimonabant (SR141716), a widely used inhibitor of CB1R activity, has been shown to act both as a CB1R antagonist and inverse agonist.29

In addition to pharmacological modulation of the ECS, the generation of various genetic rodent models has similarly advanced our understanding of its key physiological roles. Indeed, studies examining metabolic aspects of ECS function commonly utilize animals harboring specific alterations in genes encoding various ECS components. In particular, global CB1R and CB2R-deficient mice have provided valuable insight into the role of cannabinoid receptor-directed signaling with respect to both central and peripheral control of energy homeostasis.26,30,31 Furthermore, conditional tissue-specific overexpression of cannabinoid receptors, several studies have involved using mice which are genetically deficient in enzymes implicated in the synthesis or degradation of endocannabinoids, including FAAH and MAGL.33-35 It must also be noted that pharmacological interventions performed in several rodent models of obesity have unravelled important links between the ECS and various obesity-induced factors.9,36

Controlling Motivation to Eat: A Central Aspect to ECS Functionality

There is growing evidence supporting a role for the ECS in motivational aspects of feeding behavior.41 For example, central administration of either anandamide or 2-AG in rats has been found to potently stimulate appetite and promote body weight gain.41,42 Importantly, this increase in food consumption can be prevented by co-administration of CB1R blockers such as SR141716, thereby suggesting a critical role for CB1R in mediating this hyperphagic response.43 Consistent with this, mice deficient in CB1R exhibit a lean phenotype which is associated with reduced food intake.30

Furthermore, recent studies also suggest that CB2R-induced signaling may be important for the central modulation of feeding behavior. Specifically, Romero-Zerbo et al.32 recently demonstrated that selective overexpression of CB2R in the brain leads to decreased fasting-induced feeding which, over time, results in a lean phenotype. This is in accordance with a previous study showing that mice deficient in CB2R exhibit hyperphagia.31 Indeed, further work will be required to fully establish the relative contributions of central CB1R and CB2R action, which may potentially be opposing in nature, with respect to appetite regulation.

One of the key sites in the brain involved in controlling motivational aspects of eating behavior is the lateral hypothalamic area. Indeed, it has been proposed that the appetite-stimulating effects of cannabinoids may be due to their ability to induce alterations in the excitability of neurons present within this region of the brain.44 To support this, CB1R is expressed in hypothalamic neurons directly implicated in feeding behavior.30 Indeed, ECS modulation may promote changes in appetite through altering levels of neuropeptide hormones which convey appetite enhancing or suppressing effects. For example, SR141716 administration leads to reduced expression of the orexigenic neuropeptide Y hormone, while
at the same time increasing anorexigenic cocaine- and amphetamine-regulated transcript (CART) and α-melanocyte-stimulating hormone (αMSH) levels within the hypothalamic region\textsuperscript{45} (see Figure 2). An active ECS may also promote hyperphagia through the neural reward circuitry, a neuronal network interconnecting a number of cortices and/or nuclei including the medial forebrain bundle, nucleus accumbens, and prefrontal cortex.\textsuperscript{46} For example, injection of 2-AG directly into the nucleus accumbens shell of rats increases food intake in a CB1R-dependent manner.\textsuperscript{41} Therefore, endocannabinoids act centrally to stimulate appetite either by enhancing motivation to consume food, relieving some form of inhibitory control of food intake, or by enhancing reward sensation in response to eating—particularly in the pathological state of obesity.

In addition to these central effects, growing evidence suggests that ECS-mediated regulation of energy balance may also be peripherally directed. Indeed, several key observations allude to this proposition. For example, independent studies led by Colombo and Ravinet-Trillou demonstrated that diet-induced obese rats and mice rapidly develop tolerance to the appetite suppressing effects of SR141716 only after several days of treatment—despite exhibiting more prolonged weight loss in response to CB1R blockade.\textsuperscript{39,47} Second, peripheral (as opposed to central) infusion of CB1R agonists or SR141716 can promote either hyperphagia or hypophagia, respectively, in partially satiated rats.\textsuperscript{48} Endocannabinoids acting via the CB1 receptor are also involved in the regulation of peripheral sweet taste, by opposing the effects of

### Figure 1
Summary of the major pathways implicated in the synthesis and metabolism of anandamide and 2-AG. AR-PIP, arachidonic acid-containing inositol phospholipids; COX2, cyclooxygenase-2; DAG, diacylglycerol; FAAH, fatty acid amide hydrolase; MAG, monoacylglycerol; NAAA, N-acylethanolamine-hydrolyzing amidase; NAPE-PLD, N-acylphosphatidylethanolamine hydrolyzing phospholipase D; NAT, N-acyltransferase; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLC, phospholipase C; PG, prostaglandin.

### Table 1
<table>
<thead>
<tr>
<th>Name</th>
<th>Activity at CB1 (Ki in nM)</th>
<th>Activity at CB2 (Ki in nM)</th>
<th>Comments</th>
<th>References</th>
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<td>ACEA</td>
<td>1.4 ± 0.3</td>
<td>&gt;2000</td>
<td>Selective CB1 receptor agonist</td>
<td>28, 37</td>
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<td>AM251</td>
<td>7.5</td>
<td>2000–3000</td>
<td>Selective CB1 receptor antagonist/inverse agonist</td>
<td>38</td>
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<td>SR141716</td>
<td>1.8 ± 0.2</td>
<td>—</td>
<td>Selective CB1 receptor antagonist/inverse agonist</td>
<td>36, 39, 40</td>
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<td>JWH-133</td>
<td>680</td>
<td>3.4</td>
<td>Selective CB2 receptor agonist</td>
<td>26</td>
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<tr>
<td>AM630</td>
<td>5.2 × 10\textsuperscript{3}</td>
<td>31.2</td>
<td>Selective CB2 receptor antagonist/inverse agonist</td>
<td>26</td>
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<td>HU-210</td>
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<td>0.2–0.5</td>
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<tr>
<td>WIN-55,212-2</td>
<td>4.4 ± 1.3</td>
<td>1.2 ± 0.25</td>
<td>Non-selective CB1/2 receptor agonist</td>
<td>28</td>
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</tbody>
</table>

Citations refer to studies performed using the compounds listed in order to elucidate the role of cannabinoid receptors in aspects of energy metabolism.
Overview

leptin. In addition, capsaicin, an alkaloid compound which causes sensory desensitization in a specific subpopulation of peripheral neurons, has been shown to prevent hyperphagia or hypophagia in response to CB1R agonist or antagonist treatment, respectively. Therefore, together, these findings provide a basis for exploring the role of the ECS in modulating peripheral energy storage and utilization.

**FIGURE 2** Hypothesized regulatory influence of central ECS action upon feeding behavior. Two regions of the brain which have been implicated in mediating the central effects of the ECS upon feeding behavior include the hypothalamus and reward circuitry. The brain reward circuit comprises a network of cortical and subcortical neuronal components (e.g., the ventral tegmental area, nucleus accumbens and prefrontal cortex) that intercommunicate to control various cognitive aspects of goal-directed behavior. The hypothalamus is an area of the brain consisting of a small number of nuclei, including the arcuate nucleus, which play a crucial role in regulating appetite. Indeed, this is due, at least in part, to hypothalamic neurons expressing neuropeptides which promote either orexigenic or anorexigenic responses. For example, neuropeptide Y (NPY) containing neurons are found to act as potent stimulators of appetite when they become activated. In contrast, stimulated POMC/CART neurons signal to downstream neuronal pathways resulting in suppressed appetite. Importantly activating CB1R function has been suggested to activate or inhibit NPY or POMC/CART neurons, respectively, thereby resulting in an overall increase in food intake. In addition to these CB1R-mediated effects, there is also evidence to suggest that central CB2 receptor activation may convey an overall anorectic response. Furthermore, the ECS may also induce motivational aspects of feeding behavior by acting upon components of the brain reward circuit. AEA, anandamide; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; CB1R*, CB1 receptor activation; CB2R*, CB2 receptor activation.

An Active ECS: Key Adipogenic Factor?

One of the most extensively studied peripheral tissues in which the metabolic effects of ECS modulation have been investigated is adipose tissue. Consequently, a number of adipocyte-related processes have now been shown to be regulated by the ECS. First, isolated primary adipocytes from humans and rodents are known to express various ECS components including the CB1 and CB2 receptors. Furthermore, human adipose tissue has been reported to contain, bind, and metabolize both 2-AG and anandamide. In accordance with this, cannabinoid receptor-mediated signaling has been shown to modulate adipose function in several ways. For example, adipogenic differentiation and lipogenesis can be induced in response to anandamide treatment and/or CB1R stimulation. Furthermore, CB1R activation has also been demonstrated to reduce mitochondrial biogenesis in white adipose tissue from obese mice. Conversely, administering wild-type mice with SR141716 leads to the induction of a number of genes implicated in mitochondrial biogenesis and β-oxidation. The same study also demonstrates SR141716-mediated enhancement of lipolysis in white adipose tissue which is associated with a reduction in adipose mass. Together, these observations demonstrate the beneficial effects of suppressing CB1R activity as a means of improving mitochondrial oxidative capacity and enhancing the hydrolysis of triglycerides in adipocytes.
Furthermore, blocking CB1R-induced signaling can also increase production of adiponectin—an insulin sensitizing peptide hormone derived from adipose tissue. Indeed, SR141716 treatment has been shown to elevate adiponectin expression in isolated adipocytes from obese Zucker rats, as well as raising circulating levels of this adipokine in diet-induced obese mice.\(^9,\text{57}\) Several lines of evidence also support a role for the ECS in modulating obesity-induced inflammation in adipose tissue. For example, a study by the Roche group has demonstrated that inhibiting CB1R function attenuates lipopolysaccharide (LPS)-induced pro-inflammatory cytokine [tumor necrosis factor \(\alpha\) (TNF\(\alpha\)) and interleukin-6 (IL-6)] expression in human adipocytes.\(^{53}\) Indeed, this is consistent with a separate study demonstrating SR141716-mediated reductions in circulating levels of TNF\(\alpha\) in obese Zucker rats.\(^58\) However, it should be noted that despite these observed anti-inflammatory effects in response to suppressed CB1R function, whether CB1R activation alone is sufficient to induce pro-inflammatory action within this tissue is yet to be established.

Interestingly, work performed by Deveaux and co-workers suggests that CB2R activation may act to potentiate adipose tissue inflammation. Specifically, they showed that \textit{in vivo} administration of the CB2R selective agonist JWH-133 significantly increases inflammatory (e.g., TNF\(\alpha\)) gene expression in adipose tissue of mice fed either a normal or high fat diet.\(^{26}\) Importantly, the authors of this study were able to demonstrate reduced adipose tissue inflammation in CB2R-deficient mice, as well as in response to treatment with the CB2R antagonist AM630 in obese leptin deficient ob/ob mice.\(^\) It should be noted, however, that this pro-inflammatory effect of CB2R activation in adipose tissue appears somewhat paradoxical to most other inflammatory paradigms whereby CB2R activation is mostly perceived to attenuate inflammation (see review by Pacher and Mechoulam\(^59\)). Indeed, the mechanisms by which blocking CB1R/CB2R activity attenuates pro-inflammatory signaling remain poorly understood.

In addition to these observed direct effects upon adipose tissue, endocannabinoids may also regulate the function of macrophages which infiltrate adipose tissue during the obese state and convey a pro-inflammatory and insulin desensitizing response. Indeed, \textit{in vivo} cannabinoid treatment can promote macrophage infiltration into adipose tissue in rats.\(^60\) Furthermore, pre-treating human THP1 macrophages with SR141716 prevents the development of insulin resistance in adipocytes exposed to preconditioned medium from LPS-stimulated macrophages.\(^61\) Therefore, suppressing macrophage CB1R/CB2R activity may indirectly contribute toward those beneficial effects observed in adipose tissue, in particular during the pathological state of obesity. Collectively, these observations implicate adipose tissue as a key ECS target which, through adipokine action or otherwise, may further alter metabolic responses in other peripheral tissues (e.g., skeletal muscle and liver) to regulate systemic energy homeostasis.

**ECS Regulation of Skeletal Muscle Function**

Skeletal muscle is the primary site of insulin-stimulated glucose utilization and storage and therefore plays a vital role in maintaining systemic glucose homeostasis. Importantly, a number of key observations have been made which support the regulation of metabolic function by the ECS within this tissue. First, both CB1 and CB2 receptors, as well as enzymes involved in endocannabinoid synthesis and/or metabolism, have been shown to be expressed in rodent and human skeletal muscle.\(^{58,\text{62,63}}\) A study by Liu et al. was the first to demonstrate that \textit{in vivo} administration of SR141716 increases basal oxygen consumption in soleus muscle derived from ob/ob mice.\(^62\) In accordance with this, anandamide treatment of myotubes derived from obese subjects leads to repression of genes implicated in mitochondrial fatty acid oxidation (including AMPK\(\alpha1\), AMPK\(\alpha2\) and PGC-1\(\alpha\)) in a CB1R-dependent manner.\(^{63}\)

Application of SR141716 has also been found to significantly increase glucose uptake in soleus muscle isolated from ob/ob mice, as well as in cultured rat L6 myotubes.\(^{62,\text{64}}\) Furthermore, signaling cross talk between insulin and CB1R-induced pathways in skeletal muscle has been suggested based on the findings that activation or inhibition of CB1R can lead to either the impairment or enhancement of insulin action, respectively.\(^{27,\text{28,38}}\) Interestingly, a functional interaction between the insulin and CB1 receptors involving G\(\alpha\) proteins has recently been demonstrated in pancreatic \(\beta\)-cells.\(^{65}\) Indeed, in this study, Kim and co-workers show that CB1 receptor-mediated association of G\(\alpha\) with insulin receptors lead to their reduced activity. However, whether such an interaction can alter insulin-induced responses in other peripheral tissues such as skeletal muscle has not yet been reported.

Interestingly, diet-induced obese mice which are deficient in CB2R display enhanced insulin-stimulated glucose uptake in skeletal muscle relative to control wild-type animals despite exhibiting age-dependent increases in food intake, body weight, and obesity.\(^\)
Molecular Hepatic Dysfunction—Impact of the ECS

The ECS has been implicated in regulating several important aspects of metabolic function in the liver. Osei-Hyiaman et al. first demonstrated that CB1R deficiency in mice conveys a protective effect against diet-induced hepatic steatosis, which is independent of caloric intake.\textsuperscript{48} The authors of this same study also demonstrated the ability of CB1R/CB2R mixed agonist HU210 to increase hepatic fatty acid synthesis in wild-type mice, but not in liver-specific CB1R knockouts, thereby suggesting a role for the hepatic CB1R in \textit{de novo} lipogenesis.\textsuperscript{8} Importantly, this CB1R-induced lipogenic response coincides with elevated expression of hepatic genes required for fatty acid synthesis, including the transcription factor SREBP-1c and its target gene fatty acid synthase.\textsuperscript{66} Furthermore, CB1R-mediated hepatic steatosis may also be enhanced, at least partly, through inhibition of AMPK and carnitine palmitoyltransferase-1 (Cpt1), both key positive regulators of mitochondrial fat oxidation.\textsuperscript{66–68} In accordance with this, application of SR141716 alone has recently been reported to stimulate $\beta$-oxidation in cultured mouse liver explants.\textsuperscript{69}

Treatment of isolated primary hepatocytes with 2-AG has also been shown to elevate hepatic glucose production, concomitant with induced gluconeogenic gene expression.\textsuperscript{70} Importantly, this 2-AG-mediated response was found to be attenuated by co-application of SR141716, thereby implicating a key role for CB1R in ECS-induced hepatic gluconeogenesis.\textsuperscript{70} Furthermore, mice genetically deficient in the anandamide degrading enzyme FAAH display a significant elevation in fasted hepatic glucose production, despite having increased fasted plasma insulin levels.\textsuperscript{33} Therefore, excessive ECS activity transduced through hepatic CB1R may convey unfavorable effects on whole body energy metabolism through dysregulated lipogenesis and glucose production in the liver.\textsuperscript{68} In addition, various drug-metabolizing enzymes (e.g., Cytochrome P450s) expressed within the liver may also contribute to endocannabinoid degradation or metabolism. Indeed, one study performed in human liver microsomes demonstrates the ability of P450s to metabolize anandamide into oxygenated products.\textsuperscript{71} However, it remains to be determined what the relative contribution of such enzymes are in modulating endocannabinoid levels, as well as the biological effects of the resultant metabolized products.

The ECS and Gastrointestinal Tract: Making it Easier to Digest?

The ability of the ECS to modulate gut function has also been reported.\textsuperscript{72,73} For example, cannabinoid receptor agonist-induced inhibition of gastric acid secretion and intestinal motility has been demonstrated in rats and humans, respectively.\textsuperscript{74,75} Indeed, the ECS may also regulate food intake by modulating the gut–brain signaling axis.\textsuperscript{48,73,76} To support this, both CB1R and CB2R expression has been detected in gastrointestinal (GI) tract vagal afferent nerve fibers which functionally and morphologically innervate the GI tract.\textsuperscript{77,78} Importantly, intact capsaicin-sensitive afferent nerve fibers which functionally and morphologically innervate the GI tract are required for CB1R-induced hyperphagia in response to peripherally administered anandamide.\textsuperscript{48} In addition, levels of anandamide in intestinal tissue have been shown to be up- or down-regulated in response to starvation and refeeding, respectively.\textsuperscript{48,76} Interestingly, CB1R expression in the GI tract is repressed by cholecystokinin, a peptide hormone produced in the small intestine which induces a satiating effect.\textsuperscript{79} Collectively, these findings suggest that the ECS may play an important role in maintaining proper digestive capability as well as modulating satiety signaling both within the GI tract and that conveyed to the brain.
Modulation of Pancreatic Physiology by the ECS
As well as regulating tissue sensitivity toward insulin, the ECS may also act to modulate circulating levels of this important anabolic hormone. Indeed, several studies have highlighted a potential role for the ECS in modulating signaling and associated processes within the endocrine pancreas. Importantly, both CB1 and CB2 receptors as well as other ECS components are found to be expressed in the human and murine endocrine pancreas. However, studies of ECS regulation of pancreatic insulin secretion have yielded somewhat contrasting findings. For example, in vitro studies performed in islets isolated from rodents and humans have shown that 2-AG or anandamide treatment may act to either inhibit or enhance glucose-induced release of insulin depending on the experimental procedure used. Specifically, activation of CB1R and/or CB2R activity using selective agonists applied by static incubation leads to suppressed glucose-stimulated insulin secretion in mouse and human islets. In contrast, delivery of CB1R/CB2R agonists by continuous flow (dynamic perfusion culture) results in the enhancement of stimulated insulin secretion by glucose. Consistent with this, SR141716 administration has been shown to significantly increase plasma insulin levels in a rat model of obesity. Indeed, further work will be required to establish exactly how the ECS impacts upon pancreatic islet cell function and viability, particularly under those conditions which are observed during the pathophysiological state of obesity. For example, it remains to be determined whether these ECS-mediated effects upon insulin secretion are caused by alterations to glucose sensing mechanisms in pancreatic islets, or through regulation of pathways involved in mediating β-cell glucotoxicity and/or lipotoxicity.

Aberrant ECS Regulation: A Risk Factor for Human Metabolic Syndrome?
In addition to studies carried out in vitro or through the use of various rodent models of obesity, several lines of evidence also support the notion that the ECS plays a key metabolic role in humans. First, a rise in circulating levels of anandamide and 2-AG has been widely reported in human obesity. Indeed, both circulating and tissue levels of these endocannabinoids are found to correlate positively with non-esterified free fatty acid and triglyceride levels which are significantly increased in obese human subjects. Importantly, a reduction in visceral fat mass in obese men has been shown to coincide with a decline in plasma 2-AG levels, thereby demonstrating the close relationship between adiposity and circulating endocannabinoid levels. However, the exact obesity-related factors which are involved in upregulating endocannabinoid levels remain to be identified. For example, leptin, an adipose-derived hormone peptide, has been shown to suppress levels of AEA and 2-AG in both mature 3T3-F442A adipocytes and in the rat hypothalamus. However, because levels of circulating leptin increase in proportion to adiposity, this would not account for the elevated levels of endocannabinoids observed in the obese state. Therefore, it is likely that obesity-associated factors other than leptin are involved in the synthesis, metabolism, and/or release of these bioactive lipids. Indeed, other considerations such as hyperglycemia, hyperinsulinemia, and/or chronic inflammation may also be important determinants in this regard.

Key genetic evidence also supports an important role for the ECS in regulating metabolic function in humans. First, single nucleotide polymorphisms within the CB1R locus have been reported to occur at a higher frequency in obese individuals and/or those exhibiting metabolic syndrome. In addition, increased incidence of a specific missense polymorphism in the gene encoding FAAH, the enzyme involved in anandamide degradation, occurs in individuals displaying a higher body mass index. In accordance with this, adipose FAAH mRNA expression has been shown to negatively correlate with visceral fat mass in humans.

Therefore, therapeutic targeting of the ECS in humans may potentially be achieved either through modulation of cannabinoid receptor activity and/or targeting enzymes involved in endocannabinoid synthesis/degradation. Importantly, several clinical-based studies have demonstrated that pharmacological blockade of CB1R activity promotes favorable effects with respect to a number of metabolic markers including reduced body weight as well as improved glucose and lipid profiles. Indeed, this may be driven, at least in part, through enhanced fat oxidation and/or alleviation of low grade inflammation. Unfortunately, as in the documented case of SR141716 (trade name Acomplia), such CB1R-directed treatments can also result in mood-related side effects. Despite this, efforts are being made to develop alternative CB1R blockers which do not convey such adverse psychiatric secondary effects, and would therefore provide a safer yet effective strategy for treating obesity and associated metabolic dysfunction. One such example is the CB1R neutral antagonist AM6545 which has also been shown
to promote reductions in body weight. Similar to SR141716, it is found to significantly improve glucose and lipid profiles in mice. However, in contrast to the inverse agonist, it can do so while exhibiting markedly reduced brain penetrance. Nevertheless, the relatively high CB1R abundance in the brain, combined with the low receptor occupancy required to convey a physiological response, provides a rationale for a comprehensive assessment of the potential adverse risks associated with such potential anti-obesity drugs.

Alternatively, it may be possible to co-administer drugs which act to augment the beneficial metabolic effects of CB1R blockade. For example, studies performed in mice have shown that the weight reducing effects of SR141716 can be enhanced by either genetic or pharmacological blockade of type opioid G-protein coupled receptors, or through co-treatment with the gut hormones oxyntomodulin or peptide YY3-36. Importantly, a recent study by Verty and co-workers demonstrates that co-administration of a melanin-concentrating hormone receptor (MCHR) antagonist can augment SR141716-mediated reduction in body weight of diet-induced obese mice, while normalizing SR141716-induced behavioral changes. Consequently, the development of such combinatorial therapeutic approaches could result in lower doses of SR141716 (or other CB1R blockers) being required to convey a weight reducing effect, thereby minimizing the occurrence of any unfavorable side effects.

It is also important to highlight the potential ability of non-direct ECS targeting drugs to regulate endocannabinoid levels. For example, non-steroidal anti-inflammatory drugs are known to inhibit the activity of cyclooxygenase 2 (COX2), an enzyme implicated in the degradation of anandamide and 2-AG. Interestingly, this class of drug has been shown to alter cannabinoid receptor-induced responses. Furthermore, 2-AG and anandamide can also be converted into prostaglandins via COX2 activity. Importantly, these bioactive eicasanoids have been demonstrated to modulate both inflammation and insulin sensitivity. Intriguingly, cannabinoids have not only been shown to promote prostaglandin synthesis, but structural analogs of prostaglandins have been found to exhibit agonist activity at the CB1 receptor. Therefore, one cannot rule out the possibility that some metabolic effects exerted by endocannabinoids may also be mediated through biologically derived products such as prostaglandins.

Interestingly, several rodent- and human-based studies suggest that certain dietary lipids can also regulate endocannabinoid levels. For example, maintaining obese Zucker rats or high fat fed mice on a diet supplemented with long chain ω3 polyunsaturated fatty acids (PUFAs) has been shown to significantly reduce levels of 2-AG and/or anandamide in visceral adipose tissue, skeletal muscle, and liver. Similarly, a diet enriched with krill oil, a rich source of ω3 PUFAs, has also been demonstrated to lower circulating concentrations of 2-AG in obese humans. However, whether the ability of ω3 PUFAs to suppress endocannabinoid levels accounts for their beneficial effect in alleviating a number of factors associated with metabolic syndrome remains unclear.

**Alternative ECS Molecular Targets: New Players in Energy Homeostasis?**

As well as mediating their actions through CB1R and CB2R, some compounds have been shown to promote their cannabinimetic responses by targeting non-CB1/CB2 receptors, including the G-protein coupled receptor GPR55. Importantly, emerging evidence suggests that atypical cannabinoids and/or GPR55 may also have a role in modulating energy balance and metabolism. For example, recent work by Moreno-Navarrete et al. has shown that GPR55 expression in human visceral adipose tissue correlates positively with adiposity. Furthermore, increased circulating levels of 1-α-lysophosphatidylinositol (LPI), an endogenous lipid-derived activator of GPR55, have been reported in obese and diabetic individuals.

In one particular study of feeding behavior in rats, acute central administration of the atypical cannabinoid and putative GPR55 agonist O-1602 was found to increase food intake. Furthermore, the same study also demonstrated that subchronic i.c.v. administration of O-1602 promotes increased body fat mass. However, there was no requirement for GPR55 in mediating these effects of O-1602. In contrast, Romero-Zerbo et al. have shown that O-1602-mediated activation of GPR55 can lead to the enhancement of glucose-stimulated calcium transients and insulin secretion in isolated rat pancreatic islets. Consistent with this, rats treated acutely with O-1602 display increased plasma insulin levels and improved glucose tolerance. Together, these initial studies implicate GPR55 as a potential candidate biomarker and therapeutic target for obesity-related metabolic disorders. Also, given that SR141716A is reported to be a weak GPR55 agonist, it is possible that some of the effects of this compound on food intake and energy metabolism may involve modulation of GPR55 activity as well as CB1R.
**FIGURE 3** | Involvement of the endocannabinoid system (ECS) in diabetes-induced complications. The ECS has been implicated in the development of several diabetes-associated complications. In particular, genetic or pharmacological blockade of CB1 receptor function has been shown to ameliorate various physiological processes associated with peripheral neuropathy, nephropathy, retinopathy, atherosclerosis, and cardiac dysfunction. On the basis of previous studies performed in animal models of such diabetes-induced complications or otherwise, the arrows (↑/↓) indicate whether the processes associated with each complication are likely to be up- or down-regulated, respectively, in response to CB1R activation.

**FIGURE 4** | The effects of an overactive ECS on metabolic processes within various peripheral tissues. Arrows (↑/↓) depict whether a particular process is enhanced or suppressed, respectively. Any described effect of ECS activation on a particular process which is hypothesized is marked with a question mark (based on for example evidence demonstrating the opposite effect in response to ECS inhibition/suppression or otherwise). Also highlighted is whether the process is likely to be mediated through CB1R, CB2R, or GPR55.
blockade. Furthermore, endocannabinoids and their derivatives have also been shown to modulate the activity of alternative molecular targets such as the PPARs and GPR18. Indeed, further work will be required to establish the exact role, if any, of such receptors in ECS-regulated metabolic control.\textsuperscript{19,112}

**Involvement of the ECS in Diabetes-Related Complications**

In addition to regulating physiological processes which are directly implicated in energy homeostasis, there is increasing evidence to support a role for the ECS in the pathogenesis of secondary complications associated with obesity and diabetes. Such disorders include peripheral neuropathy, kidney disease (nephropathy), retinopathy, artherosclerosis, and cardiac dysfunction. Importantly, activating or blocking CB1 receptor function has been shown to either promote or ameliorate a number of these diabetes-induced conditions\textsuperscript{1–6} (see Figure 3). Indeed, it has been suggested that CB1R-mediated regulation of inflammation, generation of reactive oxygen species, and/or cell survival may underpin some of these responses. However, whether these effects are due to alterations in systemic metabolic status or through direct actions on the tissues implicated remains to be further explored.

**CONCLUSION**

It is becoming widely recognized that the ECS may play a key role in modulating energy balance and expenditure, driven in large part through its ability to promote pleiotropic effects at both central and peripheral target sites (see Figures 2 and 4). Indeed, overactivation of CB1R-induced signaling has been shown to result in suppressed metabolic capacity and an associated net anabolic response. Conversely, blocking CB1R function can mitigate a number of obesity-induced perturbations in metabolism. Furthermore, there are emerging roles for non-CB1R molecular targets (i.e., CB2R and GPR55) in ECS-directed metabolic regulation. Therefore, the accrual of ECS-induced bioenergetic dysregulation within various tissues may, over time, generate a systemic ‘metabolic storm’ which contributes toward the pathogenesis of hyperglycemia and dyslipidemia. Importantly, as research into the metabolic effects of manipulating ECS function progresses, it promises to reveal new therapeutic strategies to combat obesity-related disorders such as diabetes.

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