Peripheral modulation of the endocannabinoid system in metabolic disease

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Highlights:

- The endocannabinoid system (ECS) is dysregulated in obesity-associated diseases
- CB_1 antagonism is a potential therapeutic target for the treatment of obesity
- CB_1 antagonists have the potential for eliciting severe psychiatric side effects
- Antagonists of CB_1 that do not cross the blood–brain barrier are in development
- Peripherally restricted CB_1 antagonists are novel therapeutic targets for obesity

Teaser: This Keynote review discusses the peripheral modulation of the ECS in liver, adipose tissue, heart, skeletal muscle, gastrointestinal tract, pancreas, kidney and the immuno-inflammatory system.

Dysfunction of the endocannabinoid system (ECS) has been identified in metabolic disease. Cannabinoid receptor 1 (CB_1) is abundantly expressed in the brain but also expressed in the periphery. Cannabinoid receptor 2 (CB_2) is more abundant in the periphery, including the immune cells. In obesity, global antagonism of overexpressed CB_1 reduces bodyweight but leads to centrally mediated adverse psychological outcomes. Emerging research in isolated cultured cells or tissues has demonstrated that targeting the endocannabinoid system in the periphery alleviates the pathologies associated with metabolic disease. Further, peripheral specific cannabinoid ligands can reverse aspects of the metabolic phenotype. This Keynote review will focus on current research on the functionality of peripheral modulation of the ECS for the treatment of obesity.

Keywords: Cannabinoid receptor; endocannabinoid system; peripherally restricted

cannabinoid antagonist; obesity.

Introduction

The prevalence of metabolic disorders has increased exponentially worldwide. Metabolic diseases are the result of excessive systemic adiposity (obesity), insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Investigation of potential therapeutic treatments for metabolic disease has focused, in part, on targets that are modulated by fatty acids or their derivatives. The endocannabinoid system (ECS) is a lipid-derived signaling system [1] that can modulate energy expenditure. The most extensively characterized of the cannabinoid (CB) receptors are CB_1 and CB_2 [1]. Endogenous agonists for these receptors are synthesized on demand and degraded via cellular uptake and enzymatic hydrolysis [2] (Figure 1). Herein, we discuss the recent advances in research regarding the roles of CB_1 and CB_2 in metabolic disease, and how pharmaceutical agents that act as ligands for these receptors can be used in the prevention and treatment of metabolic diseases through specific modulation in the periphery.

CB_1 and CB_2

The ECS comprises several ligands and two main receptors: CB_1 and CB_2. CB_1 are the most abundantly expressed G-protein-coupled receptor (GPCR) in the central nervous system (CNS) [3], with elevated expression in the hippocampus, cortex, cerebellum and basal ganglia. The main physiological function of CB_1 is modulation of neurotransmission. CB_1 has also been localized in the periphery, with expression in the dorsal root ganglion, myelinated nerve fiber bundles in the skin, macrophages, mast cells, the gastrointestinal system (predominantly in the cholinergic neurons of the myenteric), spleen, tonsils, leukocytes, skeletal muscle and renal cells [4]. By contrast, CB_2 is predominately expressed in immune cells, particularly in the spleen, thymus and circulating immune cells [5]. In immune cells, CB_2 mainly regulates

immune responses and inflammation. In addition, CB_2 is also expressed in skeletal, cardiovascular and renal systems. CB_2 is also localized with low levels of expression in the CNS, mainly in the cell bodies and dendrites of the central neurons [6]. Furthermore, CB_1 and CB_2 are also expressed in osteoblasts and osteoclasts, where they stimulate bone formation and remodeling [7,8].

In addition to CB_1 and CB_2, several other proteins have been suggested to be CB receptors based on their ability to be activated by endocannabinoids or other CB ligands. These include GPR18 [9], GPR55 and GPR119 [10]. These receptors display little similarity to CB_1 and CB_2 but can be activated by *N*-arachidonoylglycine, lysophosphatidylinositol and *N*-oleoylethanolamide, respectively. However, there is no evidence that they can be activated by these ligands *in vivo*. As such, the International Union of Basic and Clinical Pharmacology Committee (IUPHAR) on Receptor Nomenclature and Drug Classification has not classified them as CB receptors and they have retained their orphan status. There is some evidence, however, that these receptors form heterodimers with CB receptors. GPR55 heterodimerizes with CB_1 [11,12] and CB_2 [13], which could have functional significance in tissues where both receptors are co-expressed. Together with other GPCRs, endocannabinoids or other CB ligands can activate transient receptor potential (TRP) channels [14], and potentiate glycine receptors [15].

CB receptor ligands

CB receptors are activated by two endogenous ligands [*N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG)], plant-derived cannabinoids [including tetrahydrocannabinol (THC)] and a range of synthetic ligands. Based on chemical structures, CB receptor agonists are subclassified into four groups. Classical CBs consist of tricyclic

dibenzopyran derivatives that are either naturally extracted compounds of cannabis or synthetic analogs of these compounds. The most widely studied naturally isolated CBs are delta-9-tetrahydrocannabinol (Δ9-THC) and Δ8-THC [16,17]. Δ9-THC has a similar affinity to AEA for CB_1; however, it displays lower efficacy than AEA at CB_2 than at CB_1 [18]. The nonclassical CBs contain bicyclic and tricyclic analogs of Δ9-THC, including 3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-4-(3-hydroxypropyl)cyclohexanol (CP55,940), an agonist with similar affinities for both CB receptors [19]. The third group of CB receptor agonists are the aminoalkylindole cannabinoids, including (*R*)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt (WIN 55212). WIN 55212 has a high affinity for both receptor subtypes, with a slightly greater affinity for CB_1 [17,20]. The fourth group, eicosanoid CBs include AEA [21] and 2-AG [22]. Among all eicosanoid CBs, 2-AG exhibits the highest intrinsic activities at CB_1 and CB_2. AEA has a lower affinity for CB receptors and acts as a partial agonist, exhibiting mixed agonist–antagonist properties at CB_1 and CB_2.

CB receptor signaling

CB receptors predominately couple to the inhibitory Gi/o G proteins, which inhibit adenylate cyclase activity and subsequently decrease intracellular cyclic AMP levels. However, as is common with many other GPCRs, pleotropic coupling of CB receptors to other effector proteins has been reported. These include activation of Gq and Gs proteins, β-arrestin recruitment, inhibition of voltage-gated calcium channels, stimulation of inwardly rectifying potassium currents and activation of mitogen-activated protein kinase (MAPK) signaling pathways, reviewed in [23]. Because CB receptors can couple or signal to multiple effector proteins, the likelihood of biased signaling to occur is increased. Biased signaling can be

defined as ligand-dependent selectivity for specific signal transduction pathways following activation of the same receptor. It is thought to occur when different ligands bind to a receptor to cause different receptor conformations, enabling the receptor to preferentially signal to one pathway over another. Biased signaling at CB_1 and CB_2 has been reported (i.e., [24,25] for CB_1 and [26,27] for CB_2). The significance of biased signaling is attractive, in that theoretically one could design a drug with fewer side effects. However, further work is warranted in the CB receptor field to delineate those coupled signaling pathways that are beneficial or detrimental following CB activation.

Central dysfunction of the ECS in metabolic disease

The ECS is well recognized to have a vital role in the regulation of eating behavior and energy homoeostasis [28–30]. In the brain, the ECS regulates food intake by modulating activity of the hypothalamus and the limbic system [31]. In the hypothalamus, endocannabinoids are released on demand after short-term food deprivation. Thereafter, the ECS transiently regulates food intake by enhancing orexigenic mediators such as ghrelin and inhibiting anorexigenic mediators, namely leptin and cholecystokinin [32–34]. Activation of CB_1 by hypothalamic administration of AEA stimulates appetite [35], whereas inhibition of CB receptors by SR141716A (rimonabant) suppresses appetite [36].

Central dysfunction in the CB receptors has also been identified in metabolic disease. CB_1 in the forebrain and in sympathetic neurons can regulate thermogenesis and energy balance [37]: conditional knockout mice lacking CB_1 in forebrain neurons result in mice with a lean phenotype that are resistant to diet-induced obesity. Furthermore, CB_1-deficient mice have increased energy expenditure [39]; and even following consumption of a high-fat diet (HFD) (49% of energy as fat) CB_1-deficient mice do not become obese [38].

Rimonabant, the most widely studied antagonist, has been shown to act as a CB_1 antagonist and inverse agonist [40,41]. Rimonabant was developed and marketed for the treatment of obesity but was withdrawn from the market in 2008 owing to severe psychological side effects. These are thought to have occurred as a result of the ability of rimonabant to cross the blood–brain barrier to target central CB_1, located in areas of the brain implicated in depression (prefontal and frontal cortex, hippocampus, cerebellum) and anhedonia (nucleus accumbens, dorsal striatum). CB_1 antagonism (CB_1 knockout mice) also results in lower levels of several neurotransmitters, including serotonin, as reviewed in [42], which are thought to contribute to the adverse side effects observed. Thus, despite the adverse events with targeting the ECS centrally, the ability of this system to modulate food intake has led to more-recent research that has investigated peripheral modulation of CB_1 as an obesity therapeutic.

Peripheral modulation of CB_1 and CB_2

Much of our understanding about the role of the CB receptors in normal physiology has come from studies focused on their activity in disease states. Crucial to the development of therapeutics targeting the peripheral CB system is an understanding about the role they take in numerous organs and systems. Importantly, several research studies have demonstrated links between the disruption of the ECS and metabolic disease. Specifically, altered expression of CB_1 and CB_2 has been identified in several tissues from obese animals (for review, see [43]). Typically, CB_1 expression is increased in obesity in a tissue-specific manner, and CB_2 expression is decreased in obesity [44,45]. Moreover, diet-induced obesity increases AEA and 2-AG concentrations in the brain and peripheral tissues of mice [46]. In a human study, circulating 2-AG levels were significantly elevated in obese compared with lean individuals

and significantly correlated with body mass index (BMI), percent body fat and visceral fat in males and females [47]. In addition, plasma 2-AG but not AEA levels were positively correlated with cardio-metabolic risk factors, including intra-abdominal adiposity in obese men [48]. A recent study found that the circulating levels of 2-AG are higher in insulin-resistant compared with insulin-sensitive obese postmenopausal women [49]. Within the periphery, the ECS modulates the production of hormones from the gut and pancreas, and controls functions within the liver, adipose tissue, heart and skeletal muscle (Figure 2) – organs that are key to the progression of metabolic disease. Thus, several researchers have proposed that the development of peripherally restricted CB receptor antagonists could yield novel and exciting therapeutics in obesity [50,51].

Endocannabinoids and leptin signaling

The adipokine leptin plays a key part in food intake, bodyweight control and metabolism. The main role of leptin is via the modulation of neuronal signaling pathways in the hypothalamus where it acts as an anorexigenic mediator of food intake, acting via the leptin receptor Ob-Rb [52]. Because cannabinoids are orexigenic, hypothalamic concentrations of cannabinoids are inversely correlated with plasma concentrations of leptin [33]. Anorexigenic proopiomelanocortin (POMC) and orexigenic neuropeptide Y (NPY) expressing neurons in the arcuate nucleus (ARC) are potential targets for the action of leptin in the regulation of feeding behavior [53]. The peripheral CB_1 antagonist JD5037 has recently been demonstrated to restore hypothalamic leptin sensitivity by activating anorexigenic POMC neurons [54], with JD5037 also demonstrated to reduce obesity by reversing leptin resistance in diet-induced obese (DIO) mice [55]. Further research has demonstrated that leptin directly inhibits endocannabinoid synthesis by reducing intracellular calcium levels and glucocorticoid-

mediated CB release [56,57]. AEA is significantly reduced in white adipose tissues (WAT) following leptin infusion in rats [58]. In isolated human cytotrophoblasts, 2-AG downregulates leptin expression, which is reversed by CB_1 and CB_2 antagonists, suggesting that the 2-AG regulation of leptin expression is dependent on CB receptors [59].

Mitochondrial modulation by CB signaling

Metabolic disturbances such as diabetes and obesity are associated with altered mitochondrial respiratory function [60,61]. Importantly, endocannabinoids have been found to modulate mitochondrial morphology and membrane permeability [62]. AEA promotes mitochondrial swelling and membrane fluidity but downregulates cytochrome release and membrane potential [63,64]. In isolated rat liver mitochondria, AEA inhibits oxidative phosphorylation by blocking F_0/F_1 ATP synthase activity [65]. In addition, endocannabinoid, phytocannabinoid and synthetic CB receptor agonists such as AEA, Δ9-THC and HU 210 reduce mitochondrial oxygen consumption in a dose-dependent manner in rat heart mitochondria [66]. This study further demonstrated that CB_1 agonists induce biphasic changes in complex I and/or complex II/III activities. In 2008, Tedesco *et al.* investigated the effects of CB_1 deletion or antagonism in W and isolated mature white adipocytes of HFD mice (60% kcal fat). They observed that CB_1 deletion or chronic rimonabant treatment countered HFD-dependent reductions in endothelial nitric oxide synthase (eNOS) expression and mitochondrial biogenesis, an effect linked to reduced adiposity and bodyweight [67]. In addition, CB_1 deletion or chronic rimonabant treatment on WAT and isolated mature white adipocytes of HFD mice countered HFD-dependent reductions in eNOS expression and mitochondrial biogenesis, an effect linked to reduced adiposity and bodyweight [68].

Recent research has demonstrated that CB_1 is localized to muscle mitochondria [69]. In the mitochondria, CB_1 activation with THC decreases mitochondria-coupled respiration, which was absent in CB_1 knockout mice [69]. Specifically, CB_1 is thought to be involved in the mitochondrial regulation of oxidative activity through enzymes responsible for the pyruvate metabolism pathway. CB_1 is also present in the membrane of mouse neuronal mitochondria ($mtCB_1$) which regulates brain mitochondrial activity and energy metabolism [70]. Recent studies have demonstrated that genetic deletion of hippocampal $mtCB_1$ prevents CB-induced reduction in memory formation, suggesting that $mtCB_1$ mediates memory processes through mitochondrial energy metabolism [71]. In addition, CB_1 in mitochondria of POMC cells regulates mitochondrial adaptations and CB-induced feeding in *Pomc-cre* mice [72].

ECS in the liver: lipid metabolism

Despite low expression, CB_1 is expressed in liver cells, including hepatic stellate cells (HSCs) [73,74] and hepatocytes [75]. CB_2 is undetectable in healthy liver tissue but is upregulated in pathological conditions such as non-alcoholic fatty liver disease (NAFLD) [76], hepatic fibrosis [77] and hepatocellular carcinoma (HCC) [78]. A recent human study demonstrated for the first time that serum levels of 2-AG, but not AEA, are significantly increased in patients with NAFLD, independent of obesity status of the patient [79].

Several studies have suggested that targeting the ECS in the liver could have benefits in obesity. In rodents consuming a HFD, there is an increase in CB_1 protein expression in purified liver plasma membranes. Further, agonism of CB_1 in mice consuming a standard chow diet elevates *de novo* lipogenesis via sterol regulatory element binding protein-1c (SREBP-1c), a lipogenic transcription factor regulating fatty acid synthase (FAS) and other lipogenic enzymes [80]. The mechanism for this is a reduction in fatty acid amide hydrolase (FAAH)

which is likely to be independent of the central modulation of the ECS. This study was supported by later work that confirmed a reduced rate of *de novo* lipogenesis in the liver of specific CB_1 knockout mice [81]. In a diet-induced obesity mouse model, CB_1 antagonism improves liver steatosis and lipid handling [82]; and in obese Zucker fa/fa rats CB_1 antagonism reverses liver steatosis and reduces steatohepatitis-associated high liver tumor necrosis factor (TNF)-α levels [83]. These findings have been supported by *in vitro* studies in hepatic cells, which demonstrate an improved lipogenesis after CB_1 antagonist treatment [84,85]. Reversal of HFD-induced hepatic steatosis and fibrosis by CB_1 antagonism is mediated by adiponectin via increasing fatty acid oxidation and reducing free fatty acid uptake into the liver [86]. Hepatic CB_1 is necessary for HFD-induced hepatic insulin resistance because hepatocyte-specific CB_1 knockout mice receiving HFD remain insulin sensitive [87]. Studies have also shown that hepatic insulin resistance induced by HFD in murine models is mediated by CB_1-dependent activation of the long-chain ceramide synthesis in liver [88]. These findings suggest hepatic CB_1 as a potential therapeutic target for obesity-associated insulin resistance.

In humans, CB_2 is increased in individuals with liver disease [76]. Several studies report that CB_2 agonists increase the extent of hepatic steatosis [89,90]. CB_2 agonist JWH133 enhanced HFD-induced hepatic steatosis in wild-type mice; however, the effect was blunted in CB_2-deficient mice [89]. An *in vitro* study demonstrated that CB_2-selective agonist AM1241 increases the degree of steatosis in oleic-acid-treated fatty hepatocytes [90]. Thus, targeting the ECS in the liver directly can modulate disease phenotype.

ECS in adipose tissue

Adipose tissue contributes to the regulation of many physiological processes, and dysfunction fundamentally underpins obesity and related co-morbidities [91]. Further, this endocrine

organ produces physiologically important proteins such as leptin, lipoprotein lipase and adiponectin [92]. Several studies suggest that endocannabinoids directly regulate lipid metabolism in adipose tissues *in vitro* [33,93]. Indeed, CB_1 is expressed in adipose tissue and elevated during adipogenesis [30,47,93]. CB_1 is expressed in epidymal adipose tissue and adipocytes and CB_1 agonists increase adipocyte lipoprotein lipase (LPL) activity dose-dependently in primary adipocyte cultures, whereas rimonabant reduces this effect [93]. Rimonabant also stimulates adiponectin expression in cultured adipocyte cells and reduces hyperinsulinemia in obese rats [44]. Interestingly, CB_1 expression and FAAH were elevated in mature human adipocytes compared with preadipocytes [94], suggesting an important yet undiscovered role for the ECS in functional adipocytes. Cable *et al.* reported a correlation between the endocannabinoid metabolizing enzyme FAAH and bodyweight in subcutaneous adipocytes in metabolically healthy humans. However, another catabolic enzyme: monoacylglycerol lipase (MAGL), does not correlate with bodyweight [95]. Thus, the relationship between bodyweight and the expression of components of the ECS in adipose tissue might not be straightforward. Furthermore, agonism of CB_1 enhances while rimonabant reduces insulin sensitivity in cultured adipocytes [96]. It is unlikely that this is via the modulation of glucose homeostasis because activation of endocannabinoids in human adipocytes promotes GLUT4 translocation and glucose uptake independently of insulin [97].

A recent study identified that peripheral antagonism of CB_1 in adipocytes enhances transdifferentiation of white adipocytes to the brown fat phenotype which would improve metabolism via enhancing thermogenesis [98]. In addition, chronic CB_1 antagonism activates brown adipose tissue (BAT) thermogenesis and enhances energy expenditure and glucose utilization in DIO mice [99].

ECS in the heart: role in cardiovascular disease

Obesity increases the risk of co-morbidities including cardiovascular disease, increasing the risk of developing ischemic heart disease [100]. Endocannabinoids have been studied in cardiac ischemia-reperfusion (I/R) injury. 2-AG and palmitoylethanolamide (PEA), but not AEA, protected the isolated rat heart against ischemia through agonism of CB_2 [101]. Other studies have supported the role for CB_2 but not CB_1 in myocardial I/R injury [102–105]. In isolated cardiomyocytes, treatment with rimonabant decreases transforming growth factor (TGF)-β1 fibrosis [106], suggesting that CB_1 antagonism does have a direct benefit to the heart.

In addition to ischemic heart disease, the ECS has been shown to play a major part in atherosclerosis [107,108]. Low-dose CB therapy reduces the progression of atherosclerosis in mice, predominantly by inhibiting macrophage recruitment [107]. Increased levels of 2-AG were reported in aortas and visceral adipose tissue in the pro-atherosclerotic model of ApoE null mice fed a high cholesterol diet, although CB_2 antagonism did not affect plaque formation [108]. However, CB_1 antagonism with rimonabant reduced atherosclerosis development in the aortic sinus in low-density lipoprotein (LDL)-receptor-deficient mice through anti-inflammatory effects [109]. In addition, rimonabant improves endothelial dysfunction by decreasing reactive oxygen species (ROS) production in the vessel wall of ApoE null mice fed a cholesterol-rich diet, although atherosclerotic plaque formation was not reduced [110].

In contrast to effects of CB_1 activation, selective agonism of CB_2 reduces atherosclerosis. For instance, the selective CB_2R agonist JWH015 reduced monocyte migration by reducing chemokine receptor expression in human cultured myocytes, which is generally upregulated in inflammation-mediated atherosclerosis [111]. Similarly, the CB_2 agonist JWH133 decreased atherosclerotic lesion formation, improved endothelial function

and reduced ROS levels in high-cholesterol-fed ApoE null mice. Interestingly, ApoE and CB_2 double knockout mice developed inflammatory cell infiltration in atherosclerotic plaques compared with ApoE null mice, suggesting a protective role of CB_2 in atherosclerosis [112]. Furthermore, *in vitro* analysis has shown that CB_2 activation reduces TNF-α-induced proliferation and migration of human vascular smooth muscle cells [113]. More recently, Netherland-Van Dyke *et al*. investigated the effects of CB receptor agonists on the development of atherosclerosis in $CB_2^{+/+}$ and $CB_2^{-/-}$ LDL receptor null mice and observed that lesional apoptosis and macrophage accumulation is CB_2 dependent [114]. These data provide strong evidence regarding the opposing roles of CB_1 and CB_2 in cardiovascular disease, suggesting selective CB_2 activation and CB_1 antagonism as an attractive target for the treatment of atherosclerosis.

ECS in the skeletal muscle: insulin sensitivity

It is now well accepted that the components of the ECS are expressed in muscle cells [115]. Specifically, CB_1, CB_2 and FAAH are expressed in human and rodent skeletal muscle [116]. The first study to investigate the role of the ECS in skeletal muscle showed that the CB_1 antagonist rimonabant increases glucose uptake in isolated soleus muscle from Lep^{ob}/Lep^{ob} mice [117]. Further, in isolated cells AEA modulates skeletal muscle oxidative pathways [115]. However, not all the effects of AEA are sensitive to CB_1 antagonism, suggesting the presence of other CB receptors [115]. CB_1 expression is increased in the soleus muscle of HFD-fed mice [34], with Lindborg *et al.* reporting a decreased CB_1 expression in the soleus of insulin-resistant obese Zucker rats compared with lean controls [118]. Similarly, CB_2 expression is decreased and MAGL expression upregulated in skeletal muscle of HFD-fed rats [119]. A recent study suggested that dietary intake rather than the presence of obesity influenced ECS activity in

skeletal muscle, because a HFD was shown to downregulate muscle CB_1 and MAGL mRNA expression in normal and obese individuals, whether obesity was present or not [120]. Further, high n-3 PUFA intake increases expression of CB_1, CB_2 and EC synthesis enzymes in quadriceps muscles [121]. This suggests that the n-3 PUFA intake controlled the expression of the ECS.

Skeletal muscle accounts for ~70–90% of total glucose disposal under post-prandial conditions [122,123]. CB_1 plays an important part in the development of insulin resistance in skeletal muscle. AEA and adipocyte conditioned medium (CM) impairs insulin-stimulated Akt (ser473) phosphorylation in a CB_1-dependent manner in cultures of skeletal muscle cells [124]. Insulin-stimulated glucose transport is significantly increased in the isolated soleus muscle following the chronic treatment of rimonabant [125]. Mechanistically, Lipina *et al.* found that the mixed CB_1/CB_2 agonist WIN 55,212-2 downregulates insulin-stimulated ERK1/2 but not Akt activation in cultured skeletal muscle cells, whereas rimonabant sensitizes Akt and ERK1/2 signaling in myotubes, suggesting a role for the ECS in regulating muscle metabolism and function [126].

ECS in the gastrointestinal tract

The role of the ECS in the gastrointestinal tract is generally associated with feeding behavior [127–129]; however, it could also play an important part in regulating gut inflammation and thus permeability. Mechoulam *et al.* first provided evidence of the ECS in the gastrointestinal tract in 1995, detecting 2-AG but not AEA in canine gut [130]. Later, Izzo *et al.* demonstrated that AEA and 2-AG were present in the mouse small intestine [131]. CB_1 is present in normal colonic epithelium, smooth muscle and the submucosal myenteric plexus, CB_1 and CB_2 are

expressed on plasma cells in the lamina propria and CB_2 was present on gut-associated macrophages [132].

The main function of CBs in the gastrointestinal tract could be via the modulation of hormones that regulate hunger, with CB_1 and CB_2 co-localized with peptides regulating appetite in the gastrointestinal tract. Ghrelin, a circulating 28 amino acid peptide, is an orexigenic and adipogenic hormone [133]. During food deprivation, ghrelin levels increase while leptin levels decrease [133]. The orexigenic effects of ghrelin are mediated by AMP-activated protein kinase (AMPK) and are associated with central and peripheral metabolic effects [134]. Tucci *et al.* demonstrated that rimonabant can inhibit the orexigenic effect of ghrelin [135] and the same group reported no orexigenic effect of ghrelin in CB_1 knockout mice, providing strong evidence for CB_1 dependence of ghrelin effects on AMPK activity [32].

Recently, Alen *et al.* reported that peripheral CB_1 antagonism with LH-21 counteracted the orexigenic effects of ghrelin in rats [136]; however the exact mechanism remains unclear, although gastric CB_1 modulates ghrelin production through a mammalian target of rapamycin (mTOR) pathway [137]. Importantly, Kola *et al.* suggested that the metabolic effect of ghrelin on AMPK in peripheral tissues is abolished in the absence of functional CB_1, involving direct peripheral and central effects [138]. In addition, the gastrointestinal-secreted anorexigenic peptide hormone cholecystokinin (CCK) is also linked with the ECS, with CCK downregulating CB_1 expression [139]. Thus, endocannabinoids could mediate satiety signaling from the gastrointestinal tract.

ECS in the pancreas

CB_1 and CB_2 are both present in the islets of Langerhans, where CB_1 localizes predominantly to α cells and CB_2 is found in α cells and insulin-containing β cells [140,141]. *In vitro*

stimulation of CB_1 in β cells enhances basal and glucose-stimulated insulin secretion [141,142]; however, CB_2 agonism lowers glucose-dependent insulin secretion [141]. Rimonabant reportedly decreases basal insulin hypersecretion in isolated obese rat islets without affecting basal secretion in islets from lean rats [143]. By contrast, Li *et al.* reported that CB_1 and CB_2 antagonists fail to inhibit insulin secretion, suggesting involvement of CB-receptor-independent pathways in effects of some cannabinoids [144]. Kim *et al*. observed that CB_1 blockade enhanced insulin receptor signaling in β cells through the insulin receptor substrate 2-Akt pathway, and increased β cell proliferation and reduced blood glucose in *db/db* mice [145]. These contrasting results regarding the effects of CB receptor agonists and antagonists on insulin secretion warrant further studies. Recently, studies have reported that peripheral blockade of CB_1 reverses macrophage infiltration in Zucker diabetic fatty (ZDF) rats and selective knockdown of macrophage CB_1 mitigates T2DM, suggesting macrophage-expressed CB_1 as a potential target for the management of T2DM [146]. The same group later generated CB_1-deficient rats on ZDF background to observe whether there is an obligatory role of CB_1 in T2DM. They have identified that CB_1-deficient ZDF rats have improved β cell function and hyperglycemia [147].

ECS in renal function

Deutsch and Chin initially proposed the presence of the ECS in the renal system in 1993, reporting amidase activity in rat kidneys [148]. Studies confirm the presence of CB_1 throughout the nephron, including within the glomerulus [149,150], arterioles [151], tubules [152], loop of Henle [153], collecting ducts [154] and interstitium [152]. The ECS could play a part in normal tubular physiology because proximal tubule cells (PCT) express CB_1 and CB_2 [155].

It is well known that obesity is associated with developing end-stage renal disease [156,157]. Several studies have explored the role of ECS in obesity-linked kidney disease [158,159]. CB_1 is elevated in kidneys from obese rats [158], and CB_2 is downregulated in the kidneys of obese rats [160]. Further, CB_1 antagonism improves renal function, presumably by a reduction in bodyweight. Jenkin *et al*. found that chronic CB_1 antagonism improves albuminuria and renal tubular structure in diet-induced obese rats [158]. The effect of CBs in renal function could be mediated through specific renal cell types. The role of CB_1 in renal proximal tubular cells (RPTCs) in obesity-induced renal dysfunction in RPTC-specific CB_1 knockout (RPTC $CB_1R^{-/-}$) mice has been recently examined. This study found that RPTC $CB_1R^{-/-}$ mice are protected from obesity-induced lipid accumulation in the kidney, kidney injury, renal inflammation and fibrosis through the liver kinase B1/AMP-activated protein kinase pathway, suggesting the specific role of RPTCs in CB_1-mediated nephropathy [159]. Furthermore, the CB_2 agonist AM1241 improves obesity-related renal dysfunction, whereas CB_2 antagonism reduces creatinine clearance and increases kidney weight leading to renal dysfunction in diet-induced obese rats [160]. Importantly, CB_2 agonism improves renal fibrosis and function, independent to any change in bodyweight [160]. Mechanistically, this could be via a reduction in circulating leptin concentrations, occurring in the absence of a reduction in bodyweight. Within the kidney, CB_2 expression is downregulated by high concentrations of albumin [161], suggesting that, under normal physiological conditions, CB_2 plays a part in protein handling by the kidney. Collectively, these studies provide strong evidence for the therapeutic potential of targeting CB_1 and CB_2 in the treatment of obesity-related renal diseases.

ECS and immunoinflammatory system dysregulation

The effects of the ECS in these different peripheral organ systems could involve modulation of the immune system and inflammation [57,162–164]. Obesity is characterized by chronic low-grade inflammation, an effect that reinforces the obesogenic phenotype (e.g., inducing insulin-resistance) and increases risk of obesity-related diseases including atherosclerosis and T2DM. These important inflammatory processes are responsive to the endocannabinoid system and CB antagonism.

The beneficial effects of CB_1 antagonism in obese patients are attributed in part to an increase of anti-inflammatory and metabo-regulatory adiponectin [165], together with adiponectin receptors [166] in peripheral tissues. Experimental studies confirm a CB_1-dependent stimulatory effect of endocannabinoids on adipose tissue adiponectin [44,167], whereas endocannabinoids act via CB_1 to suppress proinflammatory cytokines (MIP-1β and IL-7) in association with upregulation of adiponectin in adipose tissue of obese subjects [168]. Beneficial effects of CB_1 antagonism on HFD-induced hepatic steatosis and fibrosis (but not improved adiposity and glycemic control) are adiponectin-receptor-dependent in mice [86]. Vascular dysfunction and atherosclerosis in obesity could also be responsive to anti-inflammatory actions of the ECS, with activation of CB_2 shown to limit TNF-α-induced human endothelial cell activation, adhesion and transendothelial migration of monocytes [169], and CB_1 and/or CB_2 is implicated in inhibiting endothelial inflammatory responses and TNF-α-dependent vascular smooth muscle cell proliferation and migration (important in atherosclerosis) [113,170].

Circulating endocannabinoid levels appear to be modulated in disease states associated with inflammation. Proinflammatory cytokines upregulate CB_1 and CB_2 expression in whole blood and mononuclear cells [171] and CB_1 in T lymphocytes [172,173]. CB_1 and CB_2 expression can be differentially regulated in association with altered cytokine levels in

inflammatory disease models [174,175]. Coupled with generally anti-inflammatory actions of the ECS, such observations support a regulatory feedback loop between inflammatory activation and the ECS. Indeed, endocannabinoids have been shown to suppress excess inflammation in experimental models of hepatic ischemia [176,177], LPS-dependent pulmonary inflammation [178], inflammatory pain [179,180], polymicrobial sepsis [181] and multiple sclerosis [182]. This feedback control of inflammatory cell recruitment and inflammatory mediator release by the ECS, which is potentially disrupted in disease, presents a potential therapeutic target.

Activation of CB_1 and CB_2 regulates cell migration and cytokine and chemokine production, with CB_2 activation by 2-AG inhibiting migratory activities of immune cells [183,184]. AEA also inhibits production of proinflammatory cytokines, so that it reduces human monocyte interleukin (IL)-6 and IL-8 [185]; and IL-2, TNF-α and interferon (IFN)-γ from activated human T lymphocytes [186]. In T cells, CB_2 activity can inhibit proliferation and release of IL-2, TNF-α, IL-17 and IFN-γ [186], and reduce differentiation and IL-17 release in T helper cells [187]. 2-AG also inhibits chemokine-induced chemotaxis of T cells [188]. In B cells, CB_2 activity promotes homing and retention to marginal zone in T-independent immune responses [189,190], modulates immunoglobulin class switching [191] and maintains germinal center B cells in T-dependent immunity [192]. In macrophages, CB_2 inhibits production of proinflammatory cytokines including IL-6, TNF-α and high mobility group box (HMGB)1 [193].

Despite evidence of anti-inflammatory effects of endocannabinoids, there is nevertheless evidence for proinflammatory effects in settings of doxorubicin-induced cardiomyopathy [194], nephropathy [194] and experimental dermatitis [195]. Endocannabinoids can also increase activated leukocyte function, and 2-AG could play a part

in leukocyte recruitment and inflammatory mediator release [196]. Interestingly, proinflammatory effects are more consistently linked to 2-AG rather than AEA, which, coupled with lack of effect of CB receptor agonists on leukocyte function in models of inflammation, suggests these stimulatory effects of endocannabinoids could be receptor independent and involve metabolite effects.

Development of peripheral specific CB ligands

Owing to the adverse effects of centrally targeted therapeutics, emerging research has focused on ligands that do not cross the blood–brain barrier. Several therapeutics have been developed that specifically antagonize CB_1 in the periphery. For example, the non-brain-penetrant neutral CB_1 antagonist AM6545 reduces food intake and bodyweight in rodents consuming a chow diet [197] and blocks hyperphagia in western-diet-induced obese mice [198]. AM6545 also improves leptin sensitivity and reduces adiposity in DIO mice [199]. It further reduces corticosterone-induced adiposity and attenuates the metabolic phenotype induced by corticosterone [200]. Furthermore, another compound: JD5037, a peripherally restricted CB_1 inverse agonist, decreases adipose tissue leptin secretion [55], which leads to a reversal of hypothalamic leptin resistance in diet-induced obese mice. JD5037 is also found to be effective in reducing bodyweight, hyperphagia and adiposity in an obese *Magel2*-null mouse model, an established experimental model for Prader–Willi syndrome (PWS), proposing a potential strategy for the management of obesity in PWS [201]. Finally, LH-21, a neutral CB_1 antagonist with poor brain penetration, has also been shown to reduce food intake [202], decrease leptin expression in visceral adipose tissue of diet-induced obese rats [68] and block the orexigenic effect of ghrelin [136]. Thus, research using several peripherally

acting CB_1 ligands suggests its central effects do not solely control the benefit of targeting this receptor in obesity.

Recent development in the area of CB therapeutics has led to the identification of drugs that can target CB_1 in the periphery as well as CB_2. Limited investigations have demonstrated the CB_1/CB_2 dual agonist CB-13 can inhibit cardiomyocyte hypertrophy via AMPK–eNOS signaling in isolated rodent neonatal cardiomyocytes [203]. In an additional study, a peripherally restricted CB_1/CB_2 dual agonist naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (SAB378), in a whole mouse model of experimental colitis [204], inhibited colonic propulsion in CB_1 knockout mice, but not CB_2 knockout mice. Thus, these data suggest that targeting the ECS in the gastrointestinal tract is beneficial.

One significant limitation in these studies, in terms of the development of therapeutics for metabolic disease, is the observation that in obesity CB_1 is upregulated, whereas CB_2 is downregulated [158]. Therapeutics that act as dual agonists could therefore have mixed or limited efficacies. In this therapeutic area, a more relevant therapeutic would be to antagonize CB_1 and agonize CB_2. Recent investigations of the previously characterized CB_2 agonists: GW405833 [1-(2,3-dichlorobenzoyl)-5-methoxy-2-methyl-3-[2-(4-morpholinyl)ethyl]-1H-indole] and AM1710 [1-hydroxy-9-methoxy-3-(2-methyloctan-2-yl)benzo[c]chromen-6-one], demonstrate that they are indeed dual CB_2 agonists and CB_1 antagonists [205]. Although not yet investigated as an antiobesity therapeutic, AM1710 is not brain penetrant [206], which is suggestive of a potential therapeutic that warrants further investigation. Recently, a dual target peripheral CB_1 antagonist/iNOS inhibitor was reported to be effective in mitigating liver fibrosis, reducing bodyweight, hepatic steatosis and improving glucose tolerance in mice without inducing anxiety-like behavior [207,208]. A list of the emerging therapeutics is provided in Table 1.

Concluding remarks

Obesity and metabolic disease constitute a major health burden throughout the world. ECS dysfunction has been identified in several target organs where expression of the ECS is altered in metabolic disease. CB_1 is abundantly expressed in the brain, and globally targeting CB_1 leads to significant adverse outcomes. CB_2 is more abundant in the periphery, including the immune cells. Research using isolated cells in culture or tissues has demonstrated that modulation of the ECS in the periphery might be a potential therapeutic for metabolic disease. More-recent identification of peripheral specific CB ligands can reverse aspects of the metabolic phenotype. Further, dual CB ligands could be investigated as a potential therapeutic. Further work on the ECS is warranted for the targeting of metabolic disease.

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Authors Biographies

Deanne Hryciw

Deanne Hryciw is currently a senior lecturer in the Menzies Health Institute Queensland at Griffith University. She received her PhD in cellular physiology from University of South Australia. Her current research interests cover the endocannabinoid system, with a particular focus on altered peripheral systems in obesity. She is also interested in developmental programming, and the modulation of maternal dietary elements in programming offspring health. She has published over 50 peer-reviewed journal articles and referenced conference proceedings and book chapters. She has presented her research at national and international invited lectures on endocannabinoid signaling in obesity and leptin.

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Nirajan Shrestha is currently doing his PhD at Menzies Health Institute Queensland at Griffith University, Australia. He has done his MS in biomedical science from Chonbuk National University, South Korea and his BS in medical biochemistry from Pokhara University, Nepal. His current research focuses on the effect of maternal diet in offspring health. He is also interested in fatty acid metabolism, the endocannabinoid system and metabolic diseases. He has published three peer-reviewed journal articles and presented his research at different national and international conferences.

Figure legends

Figure 1. Biosynthesis, degradation and metabolism of endocannabinoids. Abbreviations: AA, arachidonic acid; AEA, *N*-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; NAPE-PLD, *N*-acyl phosphatidylethanolamine-specific phospholipase D; DAGL, diacylglycerol lipase; MAGL, monoacylglycerol lipase; AC, adenylyl cyclase; AMPK, 5' adenosine monophosphate-activated protein kinase; PKA, protein kinase A; NOS, nitric oxide synthase; FA, fatty acid.

Figure 2. Peripheral modulation of the endocannabinoid system (ECS). Abbreviations: FA, fatty acid; SREBP-1, sterol regulatory element-binding protein 1; LPL, lipoprotein lipase; GI tract, gastrointestinal tract.

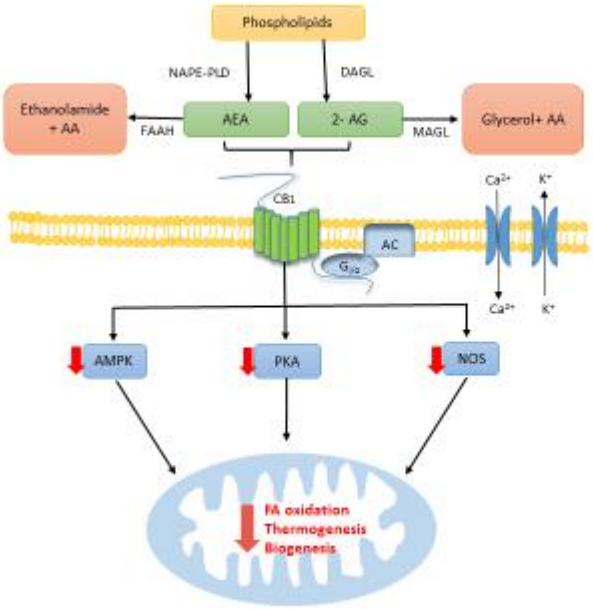
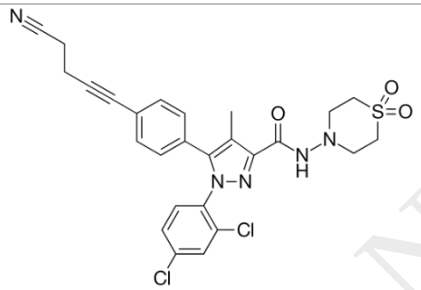
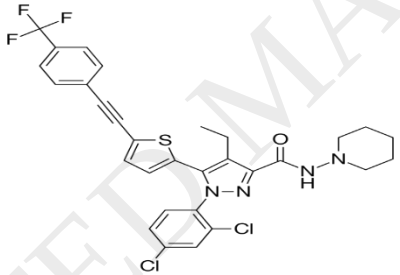
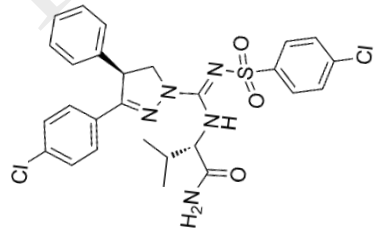
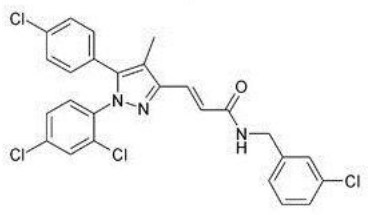
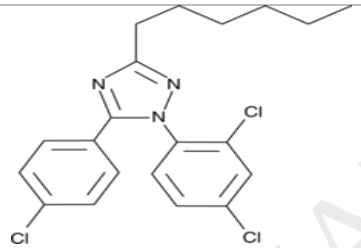
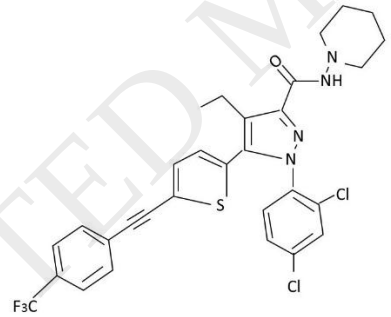
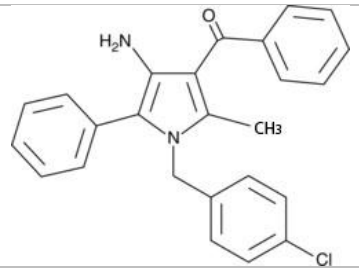
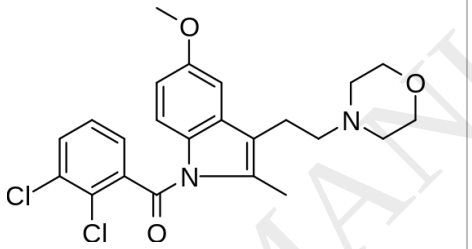
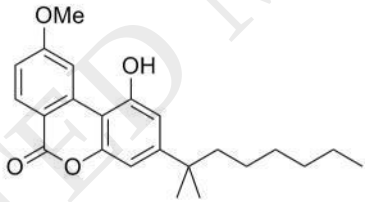
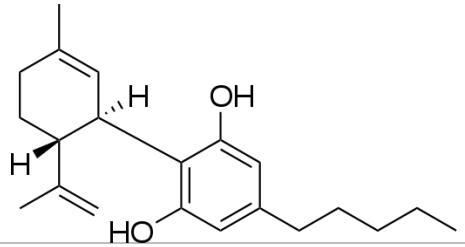


Table 1. Novel therapeutic targets modulating ECS for the treatment of obesity and metabolic disease

SN	Compound	Chemical structure	Target	Effects	Refs
1	AM6545		Peripherally restricted CB_1 neutral antagonist	Reduce food intake and bodyweight Improve dyslipidaemia by activating BAT[a]	[197]
2	TM38837		Peripherally selective CB_1 inverse agonist	Predicated to improve metabolic profile (Currently in Phase I clinical trial)	[209]
3	JD5037		Peripherally selective CB_1 inverse agonist	Attenuate glucose intolerance and insulin resistance Reverse leptin resistance	[199]
4	Compound-1		Peripheral CB_1 selective antagonist	Reduce food intake and bodyweight Decrease hepatic SREBP-1c[b]	[210]
5	LH-21		Neutral CB_1 antagonist (poor brain penetration)	Decrease food intake and bodyweight Reduce lipogenic enzymes	[202]

				Improve glucose handling	
6	BPR0912		Peripheral CB_1 antagonist	Increase β-oxidation and thermogenesis in adipose tissue	[211]
Mixed CB_1 antagonist/CB_2 agonist					
1	URB447		Mixed CB_1 antagonist/CB_2 agonist	Reduce food intake and bodyweight gain in mice	[212]

2	GW405833		Mixed CB_1 antagonist/CB_2 agonist	Not known in obesity	[27]
3	AM1710		Mixed CB_1 antagonist/CB_2 agonist	Not known in obesity	[27]
Negative allosteric modulator					
1	Cannabidiol (CBD)		Noncompetitive negative allosteric modulator of CB_1	Browning of 3T3-L1 adipocytes Inhibition of lipogenesis	[24]

[a]Brown adipose tissue.
[b]Sterol regulatory-element-binding protein 1.

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