Mitochondria: a possible nexus for the regulation of energy homeostasis by the endocannabinoid system?

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Lipina C, Irving AJ, Hundal HS. Mitochondria: a possible nexus for the regulation of energy homeostasis by the endocannabinoid system. Am J Physiol Endocrinol Metab 307: E1–E13, 2014. First published May 6, 2014; doi:10.1152/ajpendo.00100.2014.—The endocannabinoid system (ECS) regulates numerous cellular and physiological processes through the activation of receptors targeted by endogenously produced ligands called endocannabinoids. Importantly, this signaling system is known to play an important role in modulating energy balance and glucose homeostasis. For example, current evidence indicates that the ECS becomes overactive during obesity whereby its central and peripheral stimulation drives metabolic processes that mimic the metabolic syndrome. Herein, we examine the role of the ECS in modulating the function of mitochondria, which play a pivotal role in maintaining cellular and systemic energy homeostasis, in large part due to their ability to tightly coordinate glucose and lipid utilization. Because of this, mitochondrial dysfunction is often associated with peripheral insulin resistance and glucose intolerance as well as the manifestation of excess lipid accumulation in the obese state. This review aims to highlight the different ways through which the ECS may impact upon mitochondrial abundance and/or oxidative capacity and, where possible, relate these findings to obesity-induced perturbations in metabolic function. Furthermore, we explore the potential implications of these findings in terms of the pathogenesis of metabolic disorders and how these may be used to strategically develop therapies targeting the ECS.

endocannabinoid system; CB1 receptor; AEA; 2-AG; AMPK; insulin; obesity

THE ENDOCANNABINOID SYSTEM (ECS) is a ubiquitous ligand-directed signaling system involved in regulating a wide range of physiological processes, including those important for energy homeostasis (35). Two key lipid-derived molecules which act as endogenous ligands for this system are anandamide [N-arachidonoyl ethanola mine (AEA)] and 2-arachidonoylglycerol (2-AG), commonly referred to as endocannabinoids. Both AEA and 2-AG can be synthesized on demand within the plasma membrane from arachidonic acid-derived lipids. Anandamide generation from its membrane phospholipid precursor N-acylphosphatidylethanolamine (NAPE) is driven by the action of the enzyme NAPE-hydrolyzing phospholipase D (NAPE-PLD). In contrast, phospholipase C-mediated cleavage of membrane phosphatidylinositol5 gives rise to a diacylglycerol precursor whose subsequent hydrolysis (via diacylglycerol lipase activity) permits the formation of 2-AG. 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 (140).

Both AEA and 2-AG evoke cellular and physiological responses through binding and activating two distinct G protein-coupled receptors identified as the cannabinoid type 1 (CB1R) and type 2 (CB2R) receptors (23, 31, 32, 85). Indeed, various synthetic CB1R and/or CB2R agonists (e.g., CP55,940, ACEA, WIN 55,212-2, JWH-133, and HU210) have been used to provide mechanistic insight into the regulation of energy homeostasis by the ECS (Table 1) (33, 78, 87, 100, 133). Importantly, these are often applied in combination with selective receptor antagonists to determine receptor-specific responses. Such cannabinoid receptor blockers either act by competitively binding and preventing activation of a receptor by an agonist (i.e., as an antagonist) and/or function as inverse agonists through supressing spontaneous (ligand-free) receptor signaling. For example, SR141716 (also known as rimonabant) has been shown to act both as a CB1R antagonist and as an inverse agonist (Table 1) (15, 72). Notably, endocannabinoids can also mediate some of their biological effects through alternative molecular targets such as the orphan G protein-coupled receptor GPR55 or the transient receptor potential cation channel (TRPV1) (90, 127).

There is now substantial evidence supporting a role for the ECS in the modulation of energy balance and metabolism. First, various components of the ECS, including the cannabinoid receptors, their endocannabinoid ligands, and those enzymes involved in their synthesis and degradation, have been...
detected within central and peripheral tissues important for systemic energy homeostasis, including the brain, liver, adipose tissue, and skeletal muscle (37, 61, 103, 104, 136). Second, a number of studies have reported dysregulation of the ECS in the obese state (12, 38, 102). For example, circulating levels of AEA and 2-AG have been shown to be elevated in obese individuals as well as in type 2 diabetic subjects (29, 111). Third, altering CB1R- and/or CB2R-directed signaling can impact positively or negatively upon energy metabolism through the modulation of central and peripheral processes (104). Accordingly, repressing CB1R function through either genetic or pharmacological means has been shown to promote a number of beneficial metabolic effects. For example, CB1R-deficient mice are resistant to diet-induced obesity and display a lean phenotype associated with reduced food intake (28, 115). Similar anorectic responses are also observed following in vivo administration of pharmacological CB1R blockers such as SR141716 (65). These metabolic improvements can be partly explained by the ability of the ECS to regulate motivational aspects of feeding behavior (69, 106, 153).

Importantly, in addition to its effects on energy intake and appetite regulation, there is growing evidence to suggest that the ECS acts to modulate energy expenditure. In particular, independent studies led by Colombo and Ravinet-Trillou first demonstrated that diet-induced obese rats and mice rapidly develop tolerance to the appetite-suppressing effects of SR141716 after only several days of treatment, despite exhibiting more prolonged weight loss in response to CB1R blockade (27, 115). In addition, peripheral CB1R knockout models have been shown to display metabolic improvements without any significant change in food intake (103). Moreover, modulating ECS activity can lead to alterations in various key metabolic processes (26, 37, 61).

**Regulation of Energy Metabolism: the Role of Mitochondria**

Mitochondria are responsible for generating most (~90%) of cellular energy in the form of ATP. These organelles are surrounded by a smooth outer membrane and an inner membrane folded to form layers, known as cristae, which are studded with various proteins required for ATP production. The leaflets of the inner membrane are exposed to the gel-like mitochondrial matrix-harboring enzymes that catalyze a series of reactions, referred to as the tricarboxylic acid (TCA) cycle, resulting in the generation of reduced cofactors. It is the transfer of electrons from these reduced cofactors to a series of protein complexes known as the electron transport chain that establishes a proton gradient across the inner mitochondrial membrane that drives the activity of ATP synthase, the enzyme responsible for generating ATP from ADP and inorganic phosphate. Collectively, the process of generating ATP by the electron transport chain is termed oxidative phosphorylation (OXPHOS).

Importantly, perturbations in mitochondrial respiratory function have been associated with the development of a number of chronic metabolic disorders including obesity and type 2 diabetes mellitus (T2DM). This key relationship is exemplified by the finding that long-chain saturated fatty acids such as palmitate (C16:0), whose circulating levels become elevated in the obese state, act to promote insulin resistance and metabolic impairment, whereas they are predominantly degraded through mitochondrial β-oxidation (13, 70, 77). Accordingly, various indicators of reduced mitochondrial density and/or oxidative capacity have been reported in skeletal muscle and adipose tissue isolated from insulin-resistant and/or obese human subjects (51, 67, 118). For example, this includes a reduction in the activity and/or expression of carnitine palmitoyltransferase-1 (CPT-1), the rate-limiting enzyme for fatty acid entry into mitochondria, as well as components of the TCA cycle and electron transport chain coinciding with suppressed ATP synthesis (51, 53, 67, 117, 118, 134). Furthermore, lipid infusion and/or high-fat feeding in humans and rodents has been shown to reduce ATP synthesis, oxygen consumption, and oxidative phosphorylation capacity (17, 24, 134). In addition, reductions in the levels of peroxisome proliferator-activated receptor (PPAR)γ coactivator (PGC)-1α, a key transcriptional coordinator of mitochondrial biogenesis, may also contribute to free fatty acid (FFA)-induced mitochondrial dysfunction and loss of insulin sensitivity (25, 51, 77). Collectively, these observations support the idea that reduced mitochondrial density and/or oxidative capacity (associated with lipid oversupply) may restrict FFA utilization, thereby permitting the accumulation of lipotoxic intermediates such as ceramide and diacylglycerol (DAG), which have been implicated in the pathogenesis of insulin resistance (50, 68, 152, 157). It should be noted, however, that evidence obtained from a number of studies indicates that high-fat feeding can cause peripheral insulin resistance in the presence of increased mitochondrial content and/or oxidative capacity (44, 52, 89, 94, 145). Indeed, whether mitochondrial dysfunction is a consequence or cause of insulin resistance remains to be established.

**Regulation of Mitochondrial Function by the ECS**

Emerging evidence suggests that the ECS can modulate mitochondrial integrity and function. For example, exposure of

**Table 1. Synthetic modulators of cannabinoid receptor function**

<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>
</tr>
</thead>
<tbody>
<tr>
<td>AEA</td>
<td>1.4 ± 0.3</td>
<td>&gt; 2,000</td>
<td>Selective CB1 receptor agonist</td>
<td>78, 142</td>
</tr>
<tr>
<td>AM251</td>
<td>7.5</td>
<td>2,000–3,000</td>
<td>Selective CB1 receptor antagonist/inverse agonist</td>
<td>37, 61</td>
</tr>
<tr>
<td>SR141716</td>
<td>1.8 ± 0.2</td>
<td></td>
<td>Selective CB1 receptor antagonist/inverse agonist</td>
<td>58</td>
</tr>
<tr>
<td>JWH-133</td>
<td>680</td>
<td>3.4</td>
<td>Selective CB2 receptor agonist</td>
<td>75</td>
</tr>
<tr>
<td>AM630</td>
<td>5.2 × 10³</td>
<td>3.4</td>
<td>Selective CB2 receptor antagonist/inverse agonist</td>
<td>33</td>
</tr>
<tr>
<td>CP55940</td>
<td>0.5 ± 0.1</td>
<td>2.8 ± 0.4</td>
<td>Nonselective potent CB1/2 receptor agonist</td>
<td>133</td>
</tr>
<tr>
<td>HU210</td>
<td>0.1–0.7</td>
<td>0.2–0.5</td>
<td>Nonselective potent CB1/2 receptor agonist</td>
<td>4</td>
</tr>
<tr>
<td>WIN 55,212-2</td>
<td>4.4 ± 1.3</td>
<td>1.2 ± 0.25</td>
<td>Nonselective CB1/2 receptor agonist</td>
<td>47</td>
</tr>
</tbody>
</table>

Values are means ± SE. Citations refer to studies performed using the compounds listed in order to elucidate the role of the endocannabinoid system in regulating energy metabolism and/or mitochondrial function.
cells and/or isolated mitochondria to cannabinoid receptor ligands has been shown to convey deleterious effects on mitochondrial integrity, oxidative phosphorylation, and ATP production (4, 122, 123, 159). For example, studies performed in isolated rat liver mitochondria show that both AEA and 2-AG inhibit ATP synthesis at the level of F0/F1 ATP synthase when applied at low micromolar concentrations (4, 158, 159). In addition, the Cannabis sativa (marijuana)-derived cannabinoid ∆9-THC, and the synthetic cannabinoid HU210 (a mixed CB1R/CB2R agonist), have also been shown to impair mitochondrial respiratory function by reducing oxygen consumption and mitochondrial membrane potential (4). Moreover, both AEA and 2-AG have been reported to downregulate the expression of genes implicated in mitochondrial biogenesis such as PGC-1α as well as reduce mitochondrial DNA amount and oxygen consumption in mouse white adipocytes (142).

In accordance with their ability to impair mitochondrial respiratory function, endocannabinoids have also been reported to alter mitochondrial morphology and physiology (137, 151). For example, Catanzaro et al. demonstrated that AEA dose-dependently increased mitochondrial swelling, concomitant with reduced mitochondrial membrane potential and increased membrane fluidity (20). In addition, AEA has also been reported to alter mitochondrial membrane permeability (4, 20, 39, 151).

Notably, a study by Bernard et al. (8) detected CB1R within brain neuronal mitochondria, raising the possibility that this receptor may function directly within these organelles to mediate cannabinoid-induced suppression of mitochondrial respiration. Although there is ongoing debate as to what fraction of total cellular CB1R abundance is mitochondrial (which may also be cell type dependent), this same study estimated the proportion of neuronal mitochondrial CB1R abundance (relative to total cellular CB1R) to be ∼15% and potentially accounting for anything up to 30% of CB1R-dependent reduction of cell/mitochondrial respiration following AEA treatment (8). Indeed, it may be the case that, despite even a relatively low proportion of total cellular CB1R receptors present within mitochondria, their intrinsic localization and modulation may nonetheless promote significant alterations in the functionality of these organelles.

In accordance with those studies investigating the effects of CB1R activation, there is complementary evidence to suggest that suppressing CB1R-dependent signaling may improve mitochondrial oxidative capacity. For example, blockade of CB1R activity is associated with upregulated PGC-1α gene expression and increased mitochondrial biogenesis in adipocytes (61, 141). In addition, CB1R inhibition has also been shown to induce gene expression of several enzymes implicated in β-oxidation (e.g., CPT-1) and the TCA cycle (e.g., fumarase and oxoglutarate dehydrogenase) (61, 162). Consistent with these findings, treatment with SR141716 has recently been reported to stimulate β-oxidation in cultured mouse liver explants (64).

As well as the involvement of CB1R, regulation of CB2R may also play a role in modulating mitochondrial respiratory activity. For example, stimulation of CB2R using JWH133, a selective CB2R agonist, was found to convey an antiapoptotic effect during myocardial ischemia in rat hearts (75). Importantly, this protective effect coincided with the ability of JWH133 to counteract ischemia-induced loss of Δψm (mitochondrial membrane potential) and release of mitochondrial cytochrome c to the cytosol. Moreover, CB2R has also been implicated in mediating AEA-stimulated mitochondrial cation transport (165).

Collectively, these studies indicate that endocannabinoids (or exogenous cannabinoids) can act through CB1R and/or CB2R to coordinate mitochondrial function by 1) regulating mitochondrial biogenesis, 2) altering mitochondrial integrity and membrane physiology, and/or 3) modulating components of the electron transport chain. The following section discusses potential mechanisms which may underlie these responses.

The ECS-Mitochondrial Axis: Mechanistic Insights

The ECS is known to modulate a number of signaling pathways and processes that have been associated with altered mitochondrial function. Here, we discuss different ways through which the ECS may regulate mitochondrial integrity and oxidative capacity which are summarized in Figs. 1 and 2.

Involvement of toxic intracellular lipids in ECS-mediated disruption of mitochondrial function. One way that the ECS may act to impair mitochondrial integrity and function is through stimulating the generation of toxic lipid intermediates such as ceramide (26, 147). For example, both R-(+)-methanandamide, a stable analog of AEA, and WIN55,212-2, a mixed CB1R/CB2R synthetic agonist, have been shown to promote the CB1R/CB2R-dependent accumulation of ceramide in lymphoma cells, concomitant with mitochondrial depolarization (47). In accord with these findings, previous studies have demonstrated that cell treatment with exogenous ceramide promotes the release of cytochrome c, a component of the electron transport chain, from mitochondria, leading to a loss in mitochondrial membrane potential (∆ψm) and cellular ATP depletion (119, 149). Interestingly, CB2R antagonism has been reported to prevent ∆9-THC-induced mitochondrial hyperpolarization and cytochrome c release (54).

From a metabolic perspective, insulin-responsive tissues, including skeletal muscle, liver, and adipose tissue, have been shown to exhibit increased ceramide accumulation in the obese state (11, 146). This has been associated with the development of insulin resistance as well as altered mitochondrial respiratory activity (50, 132). Importantly, administration of pharmacological inhibitors of de novo ceramide synthesis [e.g., the serine palmitoyltransferase (SPT) inhibitor myriocin] has been shown to ameliorate the insulin-desensitizing effects of diet-induced obesity as well as promote improvements in whole body oxygen consumption and mitochondrial function (146, 156). Accordingly, a recent study by Cinar et al. (26) demonstrated that amelioration of diet-induced insulin resistance in response to blockade of peripheral CB1 receptors was associated with reduced de novo synthesis of long-chain ceramides in liver. Indeed, the same study was able to show that cotreatment with the SPT inhibitor myriocin was able to prevent AEA-mediated suppression of insulin-induced protein kinase B (PKB) activation in primary hepatocytes. However, the authors of that study did not relate these findings to ceramide-mediated effects on mitochondrial function. Indeed, whether obesity-associated hyperactivation of the ECS contributes toward mitochondrial dysfunction through enhanced ceramide generation remains unclear.

Notably, intracellular accumulation of ceramide can lead to inhibition of PKB, a key coordinator of numerous metabolic processes (49). Importantly, this protein kinase may also act as
a potential modulator of mitochondrial activity. For example, PKB is known to phosphorylate and inhibit the transcriptional activity of the FoxO family of transcription factors by promoting their displacement from the nucleus into the cytoplasm (155). One member of this transcription factor family, FoxO3a, has been shown to repress a large number of nuclear genes that encode for mitochondrial proteins (42). Furthermore, forced expression of FoxO3A has been reported to reduce intracellular calcium levels, the extent to which may impact either positively or negatively on mitochondrial function. It is conceivable that endocannabinoids may also be transported across the plasma membrane by transporter proteins [e.g., by the fatty acid-binding protein (FABP) family] and then subsequently act directly on target receptors residing within the mitochondrial membrane. NO, nitric oxide; NOS, nitric oxide synthase; cAMP, cyclic AMP; PKA, protein kinase A; PKB/Akt, protein kinase B; VGC, voltage-gated cation channel; FOXO, forkhead box O-class protein; TRPV1, transient receptor potential vanilloid receptor (subfamily V, member 1); AMPK, AMP-activated protein kinase; PPAR, peroxisome proliferator-activated receptor.

Role of the cAMP-PKA signaling axis in ECS-induced mitochondrial impairment. One major pathway regulated by ligand-induced CB1R activation is the cAMP-PKA (protein kinase A) signaling axis. Active CB1 receptors when coupled to G_{\text{i/o}} proteins act to inhibit adenylate cyclase, the enzyme responsible for converting ATP into 3',5'-cyclic AMP (cAMP) (82). The resulting reduction in cytoplasmic cAMP levels would ultimately suppress the activity of PKA, a cAMP-dependent protein kinase (88). To support this, AEA has been shown to inhibit forskolin-induced cAMP accumulation (41). Furthermore, CB1R-mediated decreases in cAMP and PKA activity have been reported to underlie cannabinoid receptor-induced responses in different cell types (88, 164). Conversely, the activation of PKA has been shown to mediate physiological effects of CB1R blockade (58).

Importantly, PKA has been implicated as a key modulator of mitochondrial oxidative function. For example, pharmacological inhibition of PKA has been shown to suppress cellular...
respiration in HeLa cells (1). Moreover, several studies have reported the mitochondrial localization of both soluble adenyl cyclase and PKA, the latter of which has been demonstrated to phosphorylate and regulate the activity of a number of mitochondrial proteins such as cytochrome c oxidase (1, 80, 110, 113, 125). However, it still remains to be established whether activation or inhibition of CB1R-directed signaling promotes alterations in mitochondrial oxidative metabolism through either the suppression or the enhancement of PKA-directed signaling within mitochondria, respectively. Interestingly, a recent study by Zheng et al. (163) demonstrated that CB2R stimulation leads to enhanced fatty acid oxidation in cultured muscle cells at least, in part, through activation of the PKA target CREB (cAMP response element-binding protein). This response was linked to the activation of SIRT1, a protein that functions to deacetylate and activate PGC-1α, the transcriptional coactivator involved in upregulating a number of genes that function in the complete oxidation of fatty acids (126). Indeed, the authors of that study were able to show that stimulation using a CB2R agonist led to an upregulation in the mRNA levels of CPT-1 (carnitine palmitoyltransferase-1), a key protein involved in transporting long-chain fatty acids into mitochondria for β-oxidation (163). Together, these observations suggest that the ECS, acting through CB1R and/or CB2R, may transduce the cAMP-PKA signaling axis to regulate important mitochondrial processes such as cellular respiration, fatty acid oxidation, and ATP production. Moreover, PKA activity has been reported to be reduced in visceral adipose tissue from obese individuals (84). However, whether such obesity-induced alterations in PKA are driven by ECS hyperactivation remains to be determined. Also, future studies may involve exploring the potential link between PKA activation and the protective effects of CB1R blockade against obesity-induced metabolic and mitochondrial perturbations.

Calcium signaling and ECS-mediated regulation of mitochondrial function. Calcium ions act as ubiquitous intracellular messengers that have been implicated in the regulation of numerous cellular processes, including cellular energetics. Mitochondria are able to maintain a large Ca2+ gradient across their inner membrane, thereby providing a signaling potential for this cation. Furthermore, due to their large capacity to transport and store calcium, these organelles play a vital role in regulating intracellular calcium flux (137). Importantly, elevations in mitochondrial Ca2+ have been linked to increased TCA cycle activity and NADH redox potential resulting in increased ATP synthesis and oxidative phosphorylation (46, 143). In accord with this, calcium has been reported to activate mitochondrial F1/F0 ATP synthase and subsequently increase aerobic respiration in isolated porcine heart mitochondria (46, 143).

Although there is no direct evidence for ECS modulation of mitochondrial respiratory activity through promoting changes in calcium flux, data from several studies do, however, suggest that such a link may exist. First, cannabinoid receptor-induced signaling through pertussis toxin-sensitive Gi/Go proteins can modulate the activity of various ion channels and exchangers required for cellular calcium homeostasis (55, 83, 105, 128). Indeed, activation of CB1R has been reported to stimulate the release of calcium from the endoplasmic reticulum of CB1R-transfected HEK-293 cells (16). However, ECS-mediated regulation of intracellular calcium is likely to be influenced by a number of different factors, including dose-dependent and/or tissue-specific responses (21, 83). For example, it has been shown that applying AEA to HEK-293 cells promotes the activation of vanilloid receptors, which, in turn, elevates intracellular calcium ([Ca2+]i) (112, 135). In contrast, AEA has been shown to reduce intracellular calcium concentrations by suppressing Na+/Ca2+ exchanger current in rat cardiac myocytes (74). Interestingly, AEA and 2-AG have also been reported to reduce Ca2+ dependent release of cytochrome c, a mobile electron transport protein, which, when released from mitochondria, can result in mitochondrial swelling and caspase-dependent apoptosis (20, 159). It is important to note that a functional distinction may exist between the effects of

Fig. 2. Summary of the potential ways by which ECS hyperactivation may impair mitochondrial function. A: reduced expression of transcriptional regulators of mitochondrial biogenesis [e.g., PPARγ coactivator 1α (PGC-1α)]. B: impaired respiratory chain activity. C: reduced expression/activity of carnitine palmitoyltransferase-1 (CPT-1) and AMPK, both key positive modulators of fatty acid transport and β-oxidation. D: downregulated expression of key TCA cycle components. E: stimulation of cytochrome c release. F: loss of mitochondrial membrane potential (ΔΨm). G: generation of mitochondrial reactive oxygen species (ROS).
more subtle increases in intracellular calcium that can promote improvements in mitochondrial respiratory activity, in contrast to mitochondrial calcium overload, which has been reported to reduce mitochondrial electron flow and augment mitochondrial dysfunction (130).

As yet, there have been no detailed studies investigating the metabolic effects of ECS-induced changes in mitochondrial Ca\(^{2+}\), possibly due to technical difficulties in accurately measuring mitochondrial calcium content within intact organelles. However, it is plausible that the ECS may act to alter mitochondrial capacity in part through modulating intracellular and/or mitochondrial calcium flux. Interestingly, one study by Iwabu et al. (60) reported that the ability of the anti-diabetic hormone adiponectin to enhance mitochondrial biogenesis and activity might be mediated through increases in intracellular calcium. Because CB1R activation has been shown to increase adiponectin expression (9), it is conceivable that the ECS may also indirectly regulate mitochondrial oxidative capacity by altering [Ca\(^{2+}\)], through alternative (non-ECS) pathways such as those stimulated by adiponectin.

Future studies may involve comparing the oxidative capacity of mitochondria isolated from tissues of obese diet-induced wild-type and CB1R-deficient mice and assessing how these relate to mitochondrial calcium content. Furthermore, little is known currently regarding potential synergistic effects of the ECS and other obesity-related FFA, particularly in terms of how they may impact upon mitochondrial handling of calcium.

Nitric oxide as a mediator of ECS-induced mitochondrial dysfunction. It is now well recognized that the ECS can modulate the production of nitric oxide (NO), a key signaling molecule that has been implicated in regulating mitochondrial respiration. Although a firm link between the ECS and NO in the modulation of mitochondrial function has yet to be established, emerging evidence suggests that an ECS-NO signaling axis may influence mitochondrial biogenesis and oxidative capacity. For example, activation of CB1R has been shown to repress the expression and/or activity of nitric oxide synthase (NOS), the enzyme responsible for catalyzing the synthesis of NO, in different cell types (40, 150). Importantly, enhanced NO production is associated with increased mitochondrial biogenesis, oxidative metabolism (oxygen consumption), and ATP production (98). This may, in part, be driven through its second messenger 3',5'-cyclic guanosine monophosphate (cGMP), which acts to induce PGC-1\(\alpha\)-mediated transcription of mitochondrial genes (97, 98). Consistent with this, reduced mitochondrial biogenesis as well as diminution of oxygen consumption and ATP content have been reported in various tissues of mice lacking endothelial NOS (eNOS) (97).

Interestingly, a key study by Tedesco et al. (142) demonstrated that CB1R activation by ACEA (a selective CB1R agonist) leads to the impairment of mitochondrial biogenesis in white adipocytes as a consequence of a reduction in NO-generating capacity. Importantly, the authors of that study were also able to show that CB1R activation downregulates eNOS expression and that NO donors counteract the negative effects of CB1R activation upon mitochondrial biogenesis. Conversely, inhibition of CB1R function by SR141716 upregulated a number of genes implicated in mitochondrial biogenesis and oxidative function, concomitant with increased mitochondrial DNA and mass (141). Importantly, these SR141716-induced responses were mediated through induction of eNOS, as demonstrated through their counterregulation by eNOS gene silencing (141). Consistent with these findings, both eNOS expression and mitochondrial biogenesis, which become suppressed in mature white adipocytes in response to high-fat feeding, can be restored following treatment with SR141716 (141). Interestingly, reduced serum NO levels have also been reported in obese T2DM individuals compared with nonobese controls (71). Collectively, these observations indicate that NO-dependent signaling may be a key contributor toward ECS-mediated regulation of mitochondrial biogenesis and oxidative capacity (96). However, whether the ECS acts to alter NO production, particularly in the obese and/or diabetic states, remains unclear.

AMPK: a key player in ECS-mediated regulation of mitochondrial function? AMPK is a heterotrimeric kinase consisting of a catalytic \(\alpha\)-subunit and its associated regulatory \(\beta\) and \(\gamma\)-subunits. The activity of this kinase complex can be altered in response to various cellular stimuli and regulated through different mechanisms. In particular, AMPK activity is increased in response to a fall in cellular ATP levels, for example under conditions when cells become starved of glucose. Consequently, AMPK acts to inhibit ATP-consuming pathways while stimulating those that promote ATP generation (144). In accord with this, numerous studies have reported a positive role for AMPK in the regulation of mitochondrial oxidative function and biogenesis (45, 63, 154). This includes promoting the upregulation of genes such as PGC-1\(\alpha\) and the enhancement of mitochondrial oxygen consumption and fatty acid oxidation (45, 63, 148, 154).

Importantly, evidence from several independent studies suggests that the ECS may act to modulate AMPK activity. For example, AEA exhibits a repressive effect on genes encoding AMPK\(\alpha1\) and AMPK\(\alpha2\) (AMPK catalytic subunits) in human myotubes derived from obese subjects (22). Consistent with this, reduced AMPK activity has also been reported in white adipocytes treated with the CB1R agonist ACEA (142). Conversely, both genetic repression and pharmacological blockade of CB1R have been shown to upregulate AMPK activity, concomitant with enhanced mitochondrial biogenesis and oxidative capacity within white adipose tissue from mice fed a high-fat diet (141). Therefore, modulation of the ECS, particularly through activation and/or inhibition of CB1R, may provide a means by which to negatively or positively regulate mitochondrial oxidative function, respectively, through AMPK-dependent signaling. However, further work will be required to establish the exact pathways that link the ECS to AMPK, as well as examining the role of AMPK in mediating ECS-induced metabolic effects, particularly in vivo, using relevant models of AMPK deficiency.

Alternative targets mediating endocannabinoid-regulated mitochondrial biogenesis and function. There is growing evidence that cannabinoids may also promote alterations in mitochondrial oxidative function through pathways that are not dependent on CB1R and/or CB2R (4). First, cannabinoids are able to directly bind to and regulate the activity of non-CB1R/CB2R molecular targets. One such example includes the PPAR family of nuclear receptors (101). The PPAR family consists of three distinct isoforms (PPAR\(\alpha\), PPAR\(\gamma\), and PPAR\(\delta\)), whose ligand-induced activation drives their interaction with retinoid X receptors (RXRs). The resulting PPAR-RXR heterodimers function as transcriptional regulators of lipid metabolism, energy balance, and insulin sensitivity (3, 99). Importantly, a
number of natural and synthetic cannabinoids (including AEA, 2-AG, Win55,212, Hu210, Δ9-THC) have been shown to directly bind to and regulate the activity of PPAR isoforms (14, 101, 120). Notably, PPAR activation has been shown to convey a number of beneficial effects on lipid metabolism that may be mediated through enhanced mitochondrial fatty acid oxidation (36, 48, 92, 114, 121, 139). However, despite the reported ability of ECS ligands to interact with and modulate the activity of PPARs, it still remains unclear whether a potential ECS-PPAR signaling axis acts to regulate mitochondrial oxidative function. Indeed, future work may involve applying cannabinoid receptor ligands to primary cells (e.g., hepatocytes, myotubes, adipocytes) isolated from PPAR isoform-specific gene knockout models and assessing the resulting effects on mitochondrial respiratory activity (99).

As well as targeting PPAR isoforms, endocannabinoids have also been proposed to function as ligands toward non-CB1R/CB2R G protein-coupled receptors including GPR18 and GPR55 (86, 124). As yet, however, little is known about the role, if any, of these receptors in ECS-regulated control of mitochondrial homeostasis. Interestingly, capsaicin-mediated activation of the transient receptor potential vanilloid 1 (TRPV1) cation channel, a known molecular target for AEA and 2-AG, has been reported to enhance mitochondrial oxidative capacity through upregulating the expression of genes involved in fatty acid oxidation and mitochondrial respiration (79). In accord with this, mice overexpressing TRPV1 display protection against the onset of high-fat diet-induced metabolic impairments (79). Furthermore, TRPV1 ligands have been shown to decrease mitochondrial membrane potential and oxygen consumption in isolated rat heart mitochondria (5). Indeed, this may be linked to the ability of this cation channel to modulate cytosolic calcium levels (57, 116).

ECS-mediated generation of mitochondrial reactive oxygen species. ECS-induced mitochondrial dysfunction has also been associated with increased production of reactive oxygen species (ROS). For example, both plant-derived and synthetic cannabinoid receptor agonists, as well as AEA and 2-AG, have been shown to stimulate mitochondrial ROS generation, which, in turn, may impact negatively on electron transport activity as well as promoting cytochrome c release (4, 43, 129, 160). Interestingly, a study by Fonseca et al. (43) suggested that ECS-induced generation of ROS may be driven by increased ceramide synthesis. However, whether ROS derived from mitochondria or from an alternative subcellular source is responsible for perturbing mitochondrial respiratory function by the ECS remains unclear. In addition, insulin resistance has also been associated with an increase in mitochondrial ROS emission, but whether this occurs as a result of enhanced ECS activity remains unknown, although, somewhat paradoxically, ECS stimulation has been shown to decrease proton motive force in mitochondria (4, 59, 95, 158, 159). Indeed, it may be the case that the combined effect of enhanced ROS production alongside increased ceramide generation and/or calcium overload may collectively contribute toward an overall reduction in mitochondrial oxidative capacity and/or insulin sensitivity in response to ECS hyperactivation (18, 130).

The ECS-Mitochondrial Axis: a Regulator of Pancreatic Islet Survival and Function?

Mitochondria play a vital role in coordinating various aspects of pancreatic islet function, including their ability to provide a sustainable source of metabolic messengers such as ATP, which are involved in triggering glucose-stimulated insulin secretion. Importantly, chronic exposure of pancreatic β-cells to elevated levels of glucose and/or saturated fatty acids, as observed in the obese state, can lead to deleterious effects on β-cell function. For example, hyperglycemia has been associated with increased β-cell death by apoptosis (19). As a result, insufficient insulin production and release disrupt the balance between insulin secretion and metabolic demand (109).

Amid the different mechanisms that have been proposed to cause impaired β-cell function, there is growing evidence that mitochondrial damage is a key contributor to β-cell failure in the pathogenesis of T2DM. Indeed, mitochondria in T2DM β-cells have been shown to exhibit both morphological and functional abnormalities that are not observed in control β-cells (81). Moreover, the release of proapoptotic proteins such as cytochrome c from damaged mitochondria is regarded as a key step in the initiation of β-cell apoptosis (76).

Substantial evidence now supports a role for the ECS in modulating the endocrine function of the pancreas. First, various components of the ECS, including the CB1R and CB2R, have been shown to be expressed in human and murine pancreatic islets (10, 66, 136). Importantly, studies have revealed that 2-AG and anandamide can either inhibit or potentiate insulin secretion from islets depending on the method of application. For example, stimulation of CB1R and/or CB2R activity using selective agonists applied by static incubation has reported to reduce glucose-stimulated insulin secretion from isolated mouse and human islets (66, 93). Contrary to these findings, however, there is also evidence that supports the view that cannabinoid receptor activation may act to potentiate insulin secretion when agonists are applied using a dynamic perfusion flow culture system (73). Therefore, further work will be required to establish which of these models better represents modulation by the ECS in vivo.

Currently, it remains unclear whether the effects of ECS stimulation on pancreatic cell function are linked to changes in mitochondrial integrity and/or respiratory function. However, it is plausible that CB1R/CB2R activation may act to alter mitochondrial activity, including ATP generating capacity, which in turn could impact upon the ability of β-cells to respond effectively to changes in glucose levels. Furthermore, prolonged ECS hyperactivation leading to irreversible mitochondrial damage could trigger proapoptotic signaling events and subsequent β-cell failure. Therefore, further study will be required to establish the extent to which the ECS may alter mitochondrial capacity in pancreatic islets and how this relates to their insulin secretory function, particularly in the obese state wherein elevated levels of anandamide and 2-AG have been reported in pancreatic tissue of high-fat-fed mice (136).

ECS-mediated Regulation of Mitochondrial Function: a Therapeutic Target in Humans?

Growing evidence now suggests that ECS dysregulation may be a contributing factor in the development of obesity-
related metabolic disorders in humans. First, human obesity has been shown to be associated with elevated circulating levels of anandamide and/or 2-AG as well as altered tissue expression profiles of various ECS components (12, 29, 34, 38). Importantly, because obesity-induced increases in circulating endocannabinoid concentrations have been reported to become normalized following visceral fat reduction (34), it is likely that one or more adiposity-related factors act as key determinants of systemic endocannabinoid tone. Furthermore, evidence from several genetic studies also supports an important metabolic role for the ECS in humans. For example, a higher incidence of single nucleotide polymorphisms within the CB1R gene have been reported to occur in obese subjects and/or individuals exhibiting metabolic syndrome (56, 107). In addition, an increased occurrence of a specific missense polymorphism in the gene encoding FAAH has been identified in individuals with a high body mass index (BMI) (131).

Consequently, altering ECS function in humans, either through modulation of cannabinoid receptor activity and/or by targeting enzymes involved in endocannabinoid synthesis/degradation, has been proposed as a therapeutic strategy to alleviate metabolic disorders associated with obesity. In accord with this, several clinical-based studies have demonstrated that pharmacological blockade of CB1R activity, for example by administering SR141716, is able to convey favorable metabolic responses such as reducing body weight and improving glucose and lipid profiles (30, 108). It is important to note that in the case of SR141716 these metabolic improvements were found to coincide with adverse mood-related side effects, largely due to the ability of this compound to cross the blood-brain barrier and act upon central targets. In accord with this, the ECS has been shown to drive neural progenitor proliferation, and, conversely, defective adult neurogenesis is evident in CB1R knockout mice (2, 62). Consequently, great effort has been put into developing peripherally acting CB1R antagonists such as the CB1R neutral antagonist AM6545, which has been shown to convey similar metabolic improvements as SR141716 in genetic or diet-induced obese mice (138). However, whether these beneficial responses occur as a result of improved mitochondrial oxidative capacity remains to be determined.

Importantly, a number of environmental factors such as physical activity and dietary habits can also play a fundamental role in determining metabolic health. Interestingly, studies carried out in both rodents and humans have demonstrated that consumption of certain dietary lipids can lead to a reduction in endocannabinoid levels. For example, obese Zucker rats fed krill oil, a rich source of n-3 polyunsaturated fatty acids (PUFAs), display reduced levels of AEA and 2-AG in visceral adipose tissue (7). In addition, consumption of a diet supplemented with krill oil has been shown to significantly lower circulating levels of plasma 2-AG in obese human subjects (6). In accord with these observations, PUFAs have been shown to either preserve or improve mitochondrial respiratory function (91, 161). However, as yet, it is not known to what extent dietary-induced improvements in ECS function are responsible for enhanced mitochondrial capacity.

Conclusions and Future Directions

To conclude, there is growing evidence that supports an important role for the ECS in regulating the biogenesis, integrity, and oxidative capacity of mitochondria. Collectively, the evidence presented in this review indicates that ECS activation and inhibition can convey detrimental and beneficial effects upon mitochondrial biogenesis and respiratory activity, respectively. Indeed, the highlighted studies show that ECS modulation can impact upon mitochondrial oxidative function in a number of different ways and through a variety of different mechanisms (see Figs. 1 and 2). However, it may be erroneous to assume that ECS stimulation only leads to mitochondrial dysfunction. Indeed, several studies suggest that cannabinoid receptor activation may also protect against reduced respiratory capacity under certain pathological conditions. Crucially, given the importance of maintaining mitochondrial respiratory capacity in the regulation of energy balance and homeostasis, these studies highlight the potential benefits of therapies aimed at targeting ECS components in order to counteract obesity-induced mitochondrial dysfunction.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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REFERENCES


17. Desroches J, Charron S, Bouchard JF, Beaulieu P. Endocannabinoids decrease neuropathic pain-related behavior in mice through the activation of one or both peripheral CB and CB receptors. Neuropharmacology, 2013.


Cannabinoid activation of peroxisome functionally active mitochondria in mammals. 

Endocannabinoids and obesity development - the adipose endocannabinoid signaling regulates hepatic glucose production and muscle cells. 

Review
152. Zacchigno P, D’Ornia S, Romano LL, Di Venere A, Sardanelli AM, Lorusso M. The endocannabinoid 2-arachidonoylglycerol decreases cal-


