The Endocannabinoid System and Its Relevance for Nutrition

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Abstract
Endocannabinoids bind to cannabinoid, vanilloid, and peroxisome proliferator-activated receptors. The biological actions of these polyunsaturated lipids are controlled by key agents responsible for their synthesis, transport and degradation, which together form an endocannabinoid system (ECS). In the past few years, evidence has been accumulated for a role of the ECS in regulating food intake and energy balance, both centrally and peripherally. In addition, up-regulation of the ECS in the gastrointestinal tract has a potential impact on inflammatory bowel diseases. In this review, the main features of the ECS are summarized in order to put in better focus our current knowledge of the nutritional relevance of endocannabinoid signaling and of its role in obesity, cardiovascular pathologies, and gastrointestinal diseases. The central and peripheral pathways that underlie these effects are discussed, as well as the possible exploitation of ECS components as novel drug targets for therapeutic intervention in eating disorders.
Endocannabinoids (eCBs): a group of lipid-signaling molecules that include fatty acid amides and monoacylglycerols. Anandamide (N-arachidonoylthanolamine) and 2-arachidonoylglycerol are the prototype members of the two families of endocannabinoids.

THE ENDOCANNABINOID SYSTEM

Endocannabinoids

In recent years, several natural lipids, named endocannabinoids (eCBs), have been shown to bind to and activate cannabinoid receptors (CBRs), which are the molecular targets of the Cannabis sativa active principle Δ9-tetrahydrocannabinol (Δ9-THC), N-Arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), the best studied members of fatty acid amides (FAAs) and monoacylglycerols (MAGs), respectively, are the most active eCBs as yet described (16). In addition, N-arachidonoyldopamine (NADA) has been shown to behave as a cannabimimetic compound (16), although its pharmacology is as yet poorly understood. Other endogenous FAAs are called endocannabinoid-like compounds because they do not activate CBRs but seem to have an entourage effect, i.e., they may potentiate the activity of AEA or 2-AG at their receptors by inhibiting their degradation (16). Among the latter substances are the anti-inflammatory, anticonvulsant, and antiproliferative N-palmitoylethanolamine (PEA) (47), the immunomodulator N-stearoylethanolamine, which also induces apoptosis of glioma cells (56), and notably the appetite-suppressor N-oleoylethanolamine (OEA) (29). The chemical structures of Δ9-THC, relevant eCBs, PEA, and OEA are shown in Figure 1.

Metabolic Routes

AEA and 2-AG are produced on demand through multiple biosynthetic pathways, which include key agents such as the N-acyl-phosphatidylethanolamines (NAPE)-hydrolyzing phospholipase D (NAPE-PLD) for AEA, PEA, and OEA (69), and the sn-1-specific diacylglycerol lipase for 2-AG (8). Other enzymes are also important, especially in the formation of AEA (16). The degradation of eCBs also occurs through multiple routes, which include fatty acid amide hydrolase (FAAH) (27) and monoacylglycerol lipase as major hydrolytic enzymes for AEA or 2-AG, respectively (19). These biosynthetic or degradative enzymes allow metabolic control of the endogenous tone of eCBs, and hence they regulate the biological activities of these substances. AEA, 2-AG, and their congeners, together with their target receptors and the purported endocannabinoid membrane transporters, form the endocannabinoid system.
Figure 1
Exogenous and endogenous cannabinoids. Chemical structures of the phytocannabinoid Δ⁹-THC, of biologically relevant endocannabinoids, and of the endocannabinoid-like compounds OEA and PEA are illustrated.

Molecular Targets and Signaling Pathways
AEA and 2-AG activate different signaling pathways depending on the specific receptor engaged (Table 2). To date, three CBRs have been shown to bind eCBs: type-1 (CB₁R), type-2 (CB₂R), and a purported type-3 (CB₃R or GPR55). CB₁R is mainly expressed in the central nervous system (CNS), but it is also present in peripheral tissues, including adipose tissue, liver, and skeletal muscle (64), whereas CB₂R is expressed in immune cells and in some areas of CNS (38, 94, 99). CB₃R, which shares low sequence homology (10%–15%) with the other two CB receptors, has been found in brain and spleen and couples to G₁₂ proteins in an agonist- and tissue-dependent manner (85).

Recently, the orphan G-protein coupled receptor 119 (GPR119) also has been shown to be activated by OEA, leading to stimulation of adenylyl cyclase through stimulatory G proteins (72). The activation of GPR119 could be, at least in part, responsible for the effects of OEA on food intake, a hypothesis that is supported by the localization of the receptor in brain, pancreas, and gastrointestinal tract (72).

AEA, but not 2-AG, is also an agonist of the transient receptor potential vanilloid 1 (TRPV1), which is the natural target of capsaicin, the pungent ingredient of hot peppers (16). The AEA-TRPV1 interaction occurs at a cytosolic binding site and triggers protein kinase activation, intracellular Ca²⁺ increase, mitochondrial uncoupling, and cytochrome c oxidation.

CBRs: cannabinoid receptors
Δ⁹-THC: Δ⁹-tetrahydrocannabinol
AEA: anandamide (N-arachidonoylthanolamine)
2-AG: 2-arachidonoylglycerol
PEA: N-palmitoylethanolamine
OEA: N-oleoylethanolamine
NAPE-PLD: N-acylphosphatidylethanolamines (NAPE)-phospholipase D
Table 1  Elements of the endocannabinoid system that have been best characterized so far

<table>
<thead>
<tr>
<th>Member</th>
<th>Description</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEA</td>
<td>Prototype member of fatty acid amides</td>
<td>Bioactive lipid that acts at cannabinoid and noncannabinoid receptors in the central nervous system and in the periphery</td>
</tr>
<tr>
<td>2-AG</td>
<td>Prototype member of monoacylglycerols</td>
<td>Bioactive lipid that acts at cannabinoid and noncannabinoid receptors in the central nervous system and in the periphery</td>
</tr>
<tr>
<td>EMT</td>
<td>Endocannabinoid membrane transporter</td>
<td>So far a putative entity responsible for the transport of AEA and/or 2-AG</td>
</tr>
<tr>
<td>NAPE-PLD</td>
<td>Biosynthetic enzyme</td>
<td>Partly responsible for the biosynthesis of AEA</td>
</tr>
<tr>
<td>DAGL</td>
<td>Biosynthetic enzyme</td>
<td>Mainly responsible for the biosynthesis of 2-AG</td>
</tr>
<tr>
<td>FAAH</td>
<td>Hydrolytic enzyme</td>
<td>Mainly responsible for AEA degradation</td>
</tr>
<tr>
<td>MAGL</td>
<td>Hydrolytic enzyme</td>
<td>Mainly responsible for 2-AG degradation</td>
</tr>
<tr>
<td>CB₁R</td>
<td>Cannabinoid receptor</td>
<td>Main targets of AEA and 2-AG</td>
</tr>
<tr>
<td>CB₂R</td>
<td>Cannabinoid receptor</td>
<td>Novel target of endocannabinoids</td>
</tr>
<tr>
<td>CB₃R?</td>
<td>Cannabinoid receptor</td>
<td></td>
</tr>
<tr>
<td>TRPV1</td>
<td>Vanilloid receptor</td>
<td>Target of AEA and congeners</td>
</tr>
<tr>
<td>PPARα/PPARγ</td>
<td>Peroxisome proliferator-activated receptor</td>
<td>Targets of AEA, 2-AG, and congeners</td>
</tr>
</tbody>
</table>

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol; CBRs, cannabinoid receptors; DAGL, diacylglycerol lipase; EMT, endocannabinoid membrane transporter; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acyl-phosphatidylethanolamines-hydrolyzing phospholipase D; PPAR, peroxisome proliferator-activated receptor; TRPV1, transient receptor potential vanilloid 1.

release (Table 2), all typical events of apoptosis (55). This evidence, together with the finding that TRPV1 is expressed in peripheral sensory fibers and in several nuclei of the CNS, suggests that AEA may exert a physiological control of brain functions through this receptor. In line with this, recent evidence indicates that AEA can control the level and physiological activity of 2-AG within striatum, through a TRPV1-dependent mechanism (57).

Additional targets of eCBs are the peroxisome proliferator-activated receptors (PPARs). To date, three different PPAR subtypes (α, γ, and δ), encoded by different genes and with

Table 2  Signaling pathways triggered by AEA, 2-AG, and congeners

<table>
<thead>
<tr>
<th>Receptor engaged</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB₁R/CB₂R</td>
<td>Inhibition of adenyl cyclase, type L, N and P/Q Ca²⁺ channels, nitric oxide synthase, and proapoptotic protein kinases</td>
</tr>
<tr>
<td></td>
<td>Activation of K⁺ channels, mitogen-activated protein kinase, cytosolic phospholipase A₂, phospholipase C, focal adhesion kinase, nitric oxide synthase, and sphingomyelinase/palmitoyltransferase</td>
</tr>
<tr>
<td>CB₃R?</td>
<td>Mobilization of intracellular Ca²⁺</td>
</tr>
<tr>
<td></td>
<td>Activation of RhoA</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Increase of intracellular Ca²⁺ and cytochrome c release</td>
</tr>
<tr>
<td></td>
<td>Activation of proapoptotic protein kinases</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial uncoupling</td>
</tr>
<tr>
<td>PPARα/PPARγ</td>
<td>Activation of genes involved in lipogenesis and glucose metabolism, such as C-ERβ, aP2, adiponectin, and lipoprotein lipase</td>
</tr>
</tbody>
</table>

Abbreviations: CBRs, cannabinoid receptors; PPAR, peroxisome proliferator-activated receptor; TRPV1, transient receptor potential vanilloid 1.
different tissue distribution, have been cloned (5). eCBs and some of their metabolites have been found to regulate lipid and glucose metabolism, as well as inflammatory responses, by activating PPARα or PPARγ (76).

Central and Peripheral Activities
eCBs exert their biological activity within the CNS and in peripheral tissues (Figure 2). It has been clearly documented that eCBs regulate networks and synaptic transmission through a retrograde signaling, by which they are synthesized and released from postsynaptic neurons, and bind to CB₁R in the presynaptic terminal (43). As a consequence, the release or reuptake of neurotransmitters at presynaptic terminals is modulated in a highly selective spatio-temporal manner. Such a retrograde signaling allows eCBs to modulate pain initiation, psychomotor behavior, memory, wake/sleep cycles, thermogenesis, and appetite (4, 42).

The ability of eCBs to control peripheral functions has received a great deal of attention, particularly in light of the different mechanisms underlying these effects. Indeed, it is well established that eCBs modulate reproductive processes (97), regulate cardiovascular (74) and immune functions (82), and control appetite, food intake, and energy balance (31, 76). Some of the most relevant actions of eCBs in central and peripheral tissues are summarized in Figure 3.

ENDOCANNABINOIDS IN FOOD
Arachidonic acid (AA) is a component of several eCBs, and because higher plants do not contain this fatty acid, eCBs are not found in this kingdom. It has been put forward that dark chocolate contains AEA, but this seems not to originate from the cocoa plant (18). AEA and 2-AG are found in very small amounts in animal products, in concentrations that do not have any nutritional or biological significance. However, some rodent studies suggest that there is a critical role for CB₁R in the initiation of milk suckling within the first 24 hours of birth and that the milk content of 2-AG (1–9 μg/g of lipid) may provide a stimulus to the pup to suck the nipple (66). CB₁R-null mice are, however, still able to suck milk; therefore, these studies need to be confirmed and substantiated before any conclusions are drawn.

The AEA congeners, e.g., PEA and OEA, can inhibit food intake (35) and are found in varying levels in many plant food items; the levels may even increase with storage (13). Yet, these levels are far too low to have any effect on appetite regulation. Thus, although these biological compounds are naturally found in many food items, the low concentrations are unlikely to produce any known biological effect by ingestion.

EFFECT OF DIET ON ENDOCANNABINOID LEVELS
The two major eCBs (AEA and 2-AG) are derivatives of AA, which together with linoleic acid, belong to the (n-6) family of essential fatty acids. Symptoms of (n-6)-fatty acid deficiency involve scaly skin, decreased growth, and increased transepidermal water loss, which all can be attributed to lack of linoleic acid in O-acylated ceramides of the skin (33, 34). Knock-out of the Δ⁶-desaturase, an enzyme involved in the formation of AA from dietary linoleic acid, has illustrated that mice can have a normal viability and lifespan without having any AA in the tissues, but these mice are infertile (90). Because linoleic acid (and AA) in the tissues is originating from the diet, it is not unexpected that high and low intake of polyunsaturated fat can influence tissue levels of eCBs. Thus, feeding suckling piglets with a milk formula deficient in AA decreased the brain levels of AEA and 2-AG compared to piglets consuming sow milk (6). Supplementing the milk formula with AA plus docosahexaenoic acid led to brain levels of eCBs that were not different from those of control piglets (6).

In the same study, mice were supplemented with a pharmacological dose of 0.5 weight% (1 energy%) of AA for 58 days, and these

Endocannabinoid system (ECS): an ensemble of endocannabinoids and of the proteins responsible for their metabolism, transport, and binding in central neurons and peripheral cells of the body

**TRPV1:** transient receptor potential vanilloid 1

PPARs: peroxisome proliferator-activated receptors

**Energy balance:** total body intake and expenditure of energy, which is under the control of complex and redundant neural pathways and hormonal signals that regulate feeding behavior and energy metabolism in response to the availability of nutrients in the circulation or in fat stores
mice had a ∼sixfold higher brain level of AEA (6). The diet of laboratory mice is generally very low in AA, and humans consume 100–300 mg/day (0.03–0.10 energy%), depending on the amount of meat in the diet (100). Adult rats fed for one week with 4.9 energy% AA (a high pharmacological dose) had increased levels of AA, AEA, and 2-AG in the liver and intestine but not in the brain (1), although longer dietary treatment may eventually also increase the levels in the brain. Increased brain levels of AEA, and especially 2-AG, are known to result in several behavioral effects, including analgesia, hypothermia, and hypomotility, which are associated with the classical pharmacology of cannabinoids (50). Extreme intake of long-chain (n-3) fatty acids can eventually decrease brain levels of AEA and 2-AG (98), and in other tissues their levels can also decrease to a major extent (1). The longer time required to affect brain endocannabinoid levels by dietary arachidonate and fish oils are in agreement with the fatty acid composition of the adult brain being fairly stable, whereas that of other tissues is more easily influenced by dietary fat (48). Surprisingly, brain levels of OEA, N-linoleoylethanolamine, AEA, and 2-AG were increased by high-fat diets (45 energy%) enriched in olive oil and/or safflower oil without an equivalent increase in the percentage of the corresponding fatty acids in brain total phospholipids (1). The mechanism behind the stimulatory effect of dietary unsaturated fats on brain levels of these bioactive lipids is not known. Hanus et al. (37) found that a diet with 20 weight% soybean oil for 12 days decreased brain levels of 2-AG, as did variable degrees of food restriction for 12 days. A ketogenic diet (78 weight% fat for four weeks), which also had an element of food restriction, did not decrease hippocampal levels of 2-AG, but it did reduce the level of OEA (36). Ketogenic diets are known to decrease seizure activity in epileptic individuals (28), but it is not likely that the observed change in OEA levels were associated with the observed decrease in epileptogenesis (36). Short-term fasting is known to increase levels of 2-AG and sometimes AEA (37) in areas of the brain involved in regulation of food intake, whereas feeding reduces these levels. This supports a role for eCBs as part of the system that regulates food intake in the brain.

In peripheral organs, dietary arachidonate and fish oils will, more easily than in the brain, affect eCBs levels in opposite directions (1). A high-fat diet (60 energy%) for 14 weeks increased the level of AEA in mouse liver, and this may have contributed to the observed diet-induced obesity via activation of hepatic CB1R (71). In the intestine, eCBs may contribute to reduce intestinal transit through activation of CB1R (2), whereas OEA, and perhaps other congeners such as PEA and N-linoleoylethanolamine, may have a local anorectic action that is mediated via the vagus nerve to the brain appetite center (35). It is clear that increasing the endogenous levels of OEA and congeners by transient overexpression of the NAPE-PLD will decrease across-meal satiety in rats (29). The mechanism is believed to involve intestinal activation of PPARα by OEA, which results in a nongenomic signal that translates to activation of vagal afferents reaching the brain appetite center (35). It has been found that high intake of any type of fat (45 energy%) for seven days by rats will decrease intestinal levels of OEA and congeners that all can activate PPARα (1). In acute experiments, free fatty acids infused into the intestine can have a satiety effect that may be mediated by an increased level of OEA (88), but this mechanism seems to be compromised by prolonged intake of fat (1). It is well known that the higher the fat energy% of the food, the higher the energy intake will be (22), and it is proposed that the reduction of OEA and congeners caused by high dietary fat intake may be a mechanism that can lead to overconsumption and obesity (35).

Ethanol is also a dietary constituent that has been shown to affect endocannabinoid levels, and the ECS is involved in mediating some aspects of alcohol abuse. Ethanol self-administration by rats seems to increase 2-AG level and decrease AEA level in brain interstitial fluid collected by microdialysis (11), but these aspects are outside the scope of the review.
Thus, tissue levels of eCBs, OEA, and congeners can be influenced by various dietary constituents in different directions, depending on the tissue, the dietary constituent and the time frame by which it has been consumed. In the brain appetite centers, food intake affects endocannabinoid levels, whereas in the intestine, dietary fat may compromise the anorectic effects of OEA and congeners.

CONTROL OF ENERGY BALANCE BY THE ENDOCANNABINOID SYSTEM

In mammals, an array of overlapping internal signals, acting in concert with external signals (such as olfactory and gustatory factors), governs the need for feeding. These endogenous controllers include signals released from the gastrointestinal tract after meals, such as ghrelin, cholecystokinin (CCK), and peptide YY (PYY), as well as signals more strictly related to metabolism, such as the circulating hormones insulin and leptin (64). All stimuli involved in feeding are centrally integrated by the hypothalamus, which is the key player in ensuring food intake and metabolic activity of different nutrients, thus maintaining an adequate body weight.

By now, the role played by eCBs in modulating energy balance is well established at both central and peripheral sites (Figure 3). In particular, they act by signaling through CB$_1$R, as demonstrated by the findings that, in animal models, CB$_1$R agonists are able to increase food intake, whereas the antagonist SR141716A exerts opposite effects (25). In addition, CB$_1$R knock-out mice eat less than do wild-type littermates and are resistant to diet-induced obesity (83).

Central Mechanisms

Hypothalamic neurons synthesize both catabolic [proopiomelanocortin, cocaine-amphetamine-regulated transcript (CART), and corticotrophin-releasing hormone] and anabolic (neuropeptide Y, agouti-related, and melanin-concentrating hormone) proteins, which control energy stores (64). They also possess receptors for insulin, glucocorticoids, leptin, and ghrelin, hormones that all signal changes in the nutritional state.

CB$_1$R colocalizes with many of these orexigenic or anorexigenic signals, thus suggesting that eCBs are clearly involved in the homeostatic and hedonic control of food intake and energy expenditure (40). Indeed, endocannabinoid levels increase during fasting, whereas they are reduced in satiety; accordingly, direct injection of CB$_1$R agonists into the hypothalamus exerts hyperphagic effects, whereas CB$_1$R antagonists lead to a reduction in appetite (39). The involvement of eCBs has been further demonstrated by the finding that their hypothalamic levels are pathologically elevated in obese animal models (leptin-deficient ob/ob mice and leptin receptor–deficient db/db mice), and leptin administration is able to reduce this overproduction (17). Besides their role in controlling food intake and energy balance, (endo)cannabinoids are also involved in motivational processes linked to appetite regulation, such as hunger, satiety, and specific dietary preference. Binding of eCBs to CB$_1$R enhances palatability of food: (endo)cannabinoid microinjection into the CNS (20) or FAAH inhibition (21) stimulates feeding of a high-fat/sucrose diet, an effect that can be reverted by CB$_1$R antagonists. Instead, the involvement of eCBs in dietary preference is still controversial. Escartin-Pérez et al. (25) reported that rats receiving the CB$_1$R agonist arachidonyl-2′-chloroethanolamide become hyperphagic as their prefeed period was shortened, resulting in a preference for carbohydrate-containing food. In contrast, Koch (45) found that $\Delta^9$-THC administration increased consumption of a high-fat diet.

Apart from controlling the release of peptides influencing food intake, eCBs modulate different hypothalamic networks through an additional mechanism: They retrogradely act in presynaptic neurons by inhibiting both excitatory (glutamate-mediated) and inhibitory ($\gamma$-aminobutyric acid–mediated) neurotransmitter release. Thus, the binding
of eCBs (released from postsynaptic neurons) to presynaptic CB₁R reduces corticotrophin-releasing hormone release by inhibiting glutamate release, whereas it stimulates melanin-concentrating hormone-producing neurons by inhibiting γ-aminobutyric acid release (64). Which one of the two effects is elicited depends on hormone levels, as glucocorticoids stimulate endocannabinoid synthesis and release whereas leptin inhibits synthesis and release (59).

eCBs also interact with CART signaling because CB₁R activation inhibits CART release; in particular, CART appears to be a downstream mediator of the orexigenic effects of eCBs. Indeed, FAAH knock-out mice show increased endocannabinoid levels and reduced CART levels in several hypothalamic regions involved in food intake; the effect can be reverted by CB₁R antagonist administration (70).

Finally, cross-talk between eCBs and ghrelin signaling has also been demonstrated. Ghrelin increases the endocannabinoid content in the hypothalamus and, in the meantime, eCBs enhance ghrelin release from the stomach (93). The orexigenic effect of both signals appears to be mediated by stimulation of AMP-activated protein kinase (AMPK) activity in the hypothalamus and inhibition of the activity in liver and adipose tissue. Therefore, both central and peripheral modulation of this enzyme can account for increased food intake and lipid storage triggered by eCBs and ghrelin (46).

**Peripheral Mechanisms**

The ECS is widely distributed in peripheral organs, including adipose tissue, liver, pancreas, and skeletal muscle, thus controlling body weight with a mechanism independent of caloric intake (Figure 3).

In adipose tissue, CB₁R activation leads to increased adipogenesis, either by stimulating the expression and activity of enzymes involved in fat accumulation or by promoting differentiation of preadipocytes into mature adipocytes (76). Indeed, eCBs enhance fatty acid and triglyceride biosynthesis by activating lipoprotein lipase and fatty acid synthase, as well as by increasing both basal and insulin-stimulated glucose uptake (31). In addition, they block lipolysis and fatty acid oxidation by inhibiting adenyl cyclase and AMPK activity (46, 65).

The same action can also be extended to hepatocytes, where CB₁R engagement results in activation of the steroid regulatory element-binding protein 1c transcription factor, which in turn regulates the expression of acetyl-CoA-carboxylase 1 and fatty acid synthase (71). eCBs act also in brown adipose tissue, where they inhibit thermogenesis, thus controlling energy expenditure; indeed, elevated temperature and increased uncoupling protein 1 were observed in rats after chronic administration of the CB₁R antagonist SR141716A (95). eCBs also trigger adipocyte differentiation through PPARα activation: AEA up-modulates several hallmarks of differentiation whose expression is under the control of PPARγ, including aP2, C-EBPα, Acrp30, and lipoprotein lipase (9).

The OEA-triggered appetite-suppressing effects can also be explained through binding to PPARα (29). Indeed, OEA is produced in the mucosal layer of duodenum and jejunum in feeding conditions, thus reducing food intake (88), an effect that may be compromised by dietary fat consumption (35).

Several findings indicate that eCBs are also involved in glucose tolerance, as they influence insulin secretion and glucose uptake by tissues. At present, it is not completely understood which receptor should be implicated in this phenomenon. Studies performed on mouse Langerhans islets showed that, unlike α-cells (which produce the hyperglycemic hormone glucagon), β-cells lack CB₁R, so that stimulation of CB₂R is responsible for inhibition of insulin release (41). Conversely, other studies reported that CB₁R is present in a subpopulation of mouse and human insulin-producing cells and that eCBs may act upstream by affecting one or more factors regulating hormone release (7). Matias et al. (65) found that CB₁R expression is up-regulated in insulinoma cells; however, Tharp et al. (92) failed to detect CB₁R in mouse, rat, and human α- and β-cells, whereas
they found it in δ-cells. A possible explanation for these conflicting results may be related to different methodological protocols as well as to the possibility that CB₁R expression may depend either on species specificity or on differentiation/metabolic state.

Last, but not least, the ECS might influence energy expenditure at the level of skeletal muscle. Human and rodent skeletal muscle cells express both CB₁R and CB₂R (12), but only the former seems to be involved in regulation of muscle oxidative pathways. By using specific agonists and antagonists, Cavuoto et al. (12) demonstrated that CB₁R modulates energy metabolism in skeletal muscle myotubes derived from lean and obese individuals. In particular, an increase in AMPKα1 mRNA could be seen in response to CB₁R antagonism, an effect reverted by the presence of AEA; this finding is indicative of a direct role for CB₁R on fat oxidation. The expression of pyruvate dehydrogenase kinase 4, a key regulator of the pyruvate dehydrogenase complex, decreases in response to CB₁R antagonism, suggesting that receptor blockade leads to an increased glucose flux into the citric acid cycle. Together, these results clearly indicate that, in skeletal muscle, blockade of CB₁R enhances glucose uptake and utilization (12). Furthermore, the different responsiveness showed by myotubes derived from lean and obese individuals suggests that obese individuals have an increased susceptibility to endocannabinoid activity.

**RELEVANCE OF THE ENDOCANNABINOID SYSTEM FOR OBESITY**

Several data suggest that an overactivity of the ECS promotes an obese phenotype. Indeed, endocannabinoid levels are increased before the onset of obesity (24), thus indicating that a hyperactive ECS could be a cause rather than a consequence of metabolic disorders. This evidence is also supported by epidemiological and genetic data, which show a tight association between increased adiposity in humans and faah missense polymorphism, which reduces endocannabinoid catabolism (89). Alternatively, the prolonged effects exerted by eCBs may be due to enhanced activity of NAPE-PLD and/or increased availability of the precursor AA (63). Accordingly, pharmacological blockade or genetic ablation of CB₁R reduces food intake in rodents and restores physiological metabolic parameters (such as plasma levels of lipids), thus leading to weight loss.

As reported in the previous paragraph, activation of hepatic CB₁R increases de novo lipogenesis and decreases fatty acid oxidation (71). This should contribute to the appearance of steatosis observed after high-fat diet or chronic ethanol consumption. Both conditions increase tissue levels of polyunsaturated fatty acids, which enhance endocannabinoid biosynthesis and hepatic CB₁R expression, thus improving the endocannabinoid tone into the liver. As a consequence, overactive endocannabinoid signaling elicits an increase in plasma triglyceride levels, together with a reduced apolipoprotein E-mediated triglyceride clearance (86).

Given the physiological role played by eCBs in the adipose tissue, a dysregulation of the ECS is conceivable also in the tissue of obese subjects. Nonetheless, the hyperactivity of eCBs appears to be depot specific: For example, visceral and abdominal adipose-tissue explants from obese individuals are hyperactive, whereas gluteal adipose tissue shows decreased CB₁R activity (75).

Understanding the molecular mechanisms accounting for ECS dysregulation in obesity is at present a hot topic, especially in light of the finely tuned cross-talk between eCBs and orexigenic/anorexigenic signals. Studies carried out in animals with a misfunctioning leptin signaling showed a strict relationship of leptin itself with the endocannabinoid tone in different tissues. Indeed, studies performed in ob/ob and db/db mice, as well as in fa/fa rats, demonstrate that endocannabinoid levels increase when leptin signaling is impaired. Conversely, active leptin signaling is paralleled by decreased AEA and 2-AG levels in the hypothalamus. In line with this, leptin treatment of ob/ob mice restores normal hypothalamic endocannabinoid

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**Adiposity:** the state of being obese, i.e., of having an excess body fat that has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy.
levels (17). How does leptin inversely correlate with eCBs? We have previously demonstrated that faah gene expression is enhanced by leptin and that FAAH activity and protein content in ob/ob mice are lower than those of littermates (52, 54). More recently, Thanos et al. (91) showed that leptin is also able to regulate the expression of CB1R in vivo, overall suggesting new opportunities for the control of endocannabinoid activity by modulating leptin signaling.

Finally, the ECS may also be involved in complications related to obesity, including insulin resistance and inflammation, thus contributing to the development of type 2 diabetes and atherosclerosis. Indeed, in fully differentiated adipocytes, endocannabinoid binding to CB1R inhibits the synthesis and release of adiponectin, an insulin-sensitizing and anti-inflammatory cytokine; instead, it stimulates the synthesis and release of visfatin, a cytokine with insulin-mimetic effects whose expression is regulated by insulin resistance–inducing hormones (9). The observation that ECS overactivation in obese patients is often accompanied by enhanced release of inflammatory cytokines, including interleukin-6 and tumor necrosis factor-α, suggests the possibility that a potential vicious circle can arise, thereby contributing to atherogenic inflammation and reduced insulin sensitivity.

RELEVANCE OF THE ENDOCANNABINOID SYSTEM FOR CARDIOVASCULAR DISEASES

Chronic consumption of Cannabis sativa extracts induces bradycardia and hypertension, whereas an acute supplementation triggers opposite effects. In addition, marijuana can induce classical symptoms of angina pectoris, in agreement with evidence that the use of Cannabis extracts is a risk factor for myocardial stroke in atherosclerotic patients. Like plant-derived cannabinoids, AEA induces temporary bradycardia and hypotension, followed by a short enhancement of blood pressure, and then by a permanent hypotensive effect (30). The mechanism underlying these events seems to be mediated either by indirect modulation of the sympathetic system (77) or by a direct action on myocytes and blood vessels (73). The vascular effects of (endo)cannabinoids can also be mediated by receptors other than CB1R, such as CB2R (GPR55), TRPV1, and PPARγ (49, 68), yielding vasodilatory effects that appear to be independent of NO generation. The latter event is typically triggered by CB1R agonism (53).

In the myocardium, CB1R and CB2R have a nuclear localization and, once activated by AEA, they inhibit the inositol-1,4,5-trisphosphate receptor-mediated nuclear Ca2+ release. This finding could in part explain the mechanism by which eCBs control coronary vascular tone and cardiac performance (15). Conversely, in human coronary endothelial and smooth muscle cells, CB2R, whose expression is enhanced by the proinflammatory cytokine tumor necrosis-α, decreases atherogenic events (80), whereas blockade of CB1R attenuates platelet-derived growth factor–induced cell migration and proliferation (81). Thus, CB2R may play a protective role in atherosclerosis progression by reducing the inflammatory components of atherosclerosis and by inhibiting the release of chemokines and adhesion molecules by white cells recruited into atherosclerotic plaques (67). These events are summarized in Figure 4.

The protective effect of CB2R activation has also been demonstrated in a mouse model of myocardial stroke, and an overproduction of eCBs has been observed in several forms of ischemia/reperfusion injury, especially those associated with hemorrhagic shock and acute myocardial infarction (74). CB1R may also contribute to the cardioprotective action of eCBs because AEA produced by platelets and macrophages during shock states decreases blood pressure in a CB1R-dependent manner (96). In addition, metabolites of 2-AG generated by cytochrome P450 can have hypotensive properties, since they play a role in Ca2+–induced vasodilation observed in rat mesenteric arteries (3).
Besides their beneficial effects in cardiovascular diseases (Figure 4), eCBs might exhibit prothrombotic effects. Indeed, physiological concentrations of 2-AG can activate platelets via an autocrine/paracrine mechanism, which triggers the rise of intracellular concentrations of Ca\(^{2+}\) and inositol trisphosphate and decrease of cAMP levels (51). Overall, the biological effects of eCBs within the cardiovascular system appear to be manifold.

**RELEVANCE OF THE ENDOCANNABINOID SYSTEM FOR GASTROINTESTINAL PATHOLOGIES**

A growing body of evidence suggests that the ECS plays a key role in the gastrointestinal (GI) tract, thus representing a novel therapeutic target against GI disorders spanning from emesis, diarrhea, and inflammatory bowel diseases to motility-related dysfunctions.

AEA, 2-AG, and their metabolic enzymes have been detected in the intestine of different species, including dogs, mice, rats, and humans (2). In addition, CB\(_1\)R and CB\(_2\)R have been detected in the GI tract, where they seem to have a different distribution: CB\(_1\)R is primarily localized in the enteric nervous system, whereas CB\(_2\)R is present in immune cells (62).

The distinctive expression of CB\(_1\)R in the enteric nervous system neurons accounts for the ability of eCBs to modulate physiological processes, including gastric secretion and emptying, as well as intestinal motility. For example, \(\Delta^9\)-THC supplementation has been proved to inhibit GI propulsion and motility; this inhibitory effect is achieved by counteracting histamine-stimulated gastric secretion (84) and delaying gastric emptying in a CB\(_1\)R-dependent manner (26).

In the gut, AEA has been shown to either inhibit or enhance acetylcholine release, depending on the specific receptor activated. Signal transduction triggered by CB\(_1\)R, which colocalizes with acetylcholine transferase in cholinergic neurons (14), reduces the levels of acetylcholine, which instead are increased by binding of AEA to TRPV1 (60).

Unlike CB\(_1\)R, the CB\(_2\)R-dependent signal transduction is activated under inflammatory conditions: Increased motility observed in lipopolysaccharide-treated rats can be reversed by CB\(_2\)R agonists, suggesting that activation of this receptor may be required for re-establishing normal GI motility after inflammatory stimuli (23).

For many years, *Cannabis sativa* extracts have been used in humans suffering from GI disorders and in cancer patients subjected to chemotherapy, by virtue of their antiemetic and antinausea effects (58). Indeed, activation of CB\(_1\)R can reduce gastric acid production, which is involved in the pathogenesis of gastritis, ulcerative diseases, and gastro-esophageal reflux (79). The pharmacological use of eCBs might also be helpful in the treatment of motility-related disorders: hyper- or hypocontractile states can be successfully modulated by using either agonists or antagonists of CB\(_1\)R. In line with this, cannabinoids can reduce muscle spasms in irritable bowel syndrome (87).

Recruitment of both CB\(_1\)R and CB\(_2\)R is involved in the control of inflammatory GI disorders. CB\(_1\)R activation has been shown to inhibit secretion of proinflammatory cytokines (10, 44), and experimentally induced inflammation dramatically increases in CB\(_2\)R-deficient mice whereas sustained CB\(_2\)R activation counteracts proinflammatory responses in wild-type mice (61). Similar results have been obtained in faah-deficient mice, suggesting a therapeutic approach where FAAH inhibitors are employed to treat inflammatory processes in the gut. Finally, a therapeutic use of cannabinoids has been proposed to alleviate symptoms of cancer and side effects of cancer-related treatments, such as nausea, vomiting, and loss of appetite (58). In addition, growth of colorectal carcinoma cells in vitro can be effectively inhibited by CBR agonists: The antineoplastic effect appears to be specifically mediated by CB\(_1\)R, since pharmacological blockade of this receptor, but not that of CB\(_2\)R, can revert it (32).
THERAPEUTIC EXPLOITATION OF ENDOCANNABINOID SYSTEM–ORIENTED DRUGS

In the past decade, several endocannabinoid-oriented drugs have been synthesized as next-generation therapeutics to treat diseases affecting human CNS and peripheral tissues.

Owing to its broad distribution, the endocannabinoid-degrading enzyme FAAH has represented an attractive target for the treatment of human disease conditions, from spontaneous abortion, headache, and Huntington’s disease to anxiety-related disorders (27). The most promising FAAH inhibitor seems to be URB597 (also named KDS-4103), which represents an innovative antidepressant (Figure 5). In rodents, intraperitoneal administration of URB597 elicits anxiolytic effects, which are prevented by treatment with CB$_1$R antagonists. In the context of food intake, URB597 might be a potential alternative treatment for vomiting and nausea in patients subjected to chemotherapy who do not respond to currently available antiemetic drugs (78).

Compounds able to antagonize the binding of eCBs to their receptors have been the subject of large synthetic programs in many pharmaceutical companies. The best known antagonist of CB$_1$R is SR141617A, also called rimonabant (Figure 5). Rimonabant selectively blocks CB$_1$R within the brain, as well as in peripheral tissues such as fat cells, liver, and muscle. Rimonabant is likely to be exploited to cure eating disorders. Indeed, as described above, CB$_1$R stimulation enhances lipogenesis and inhibits glucose and fatty acid oxidation with a mechanism requiring a finely tuned cross-talk among different organs (e.g., hypothalamus, adipocytes, hepatocytes, endocrine pancreas, and skeletal muscle). On the basis of these data, rimonabant has been tested on humans as an antiobesity drug, and four large multicenter randomized Phase III trials have been published: RIO (Rimonabant In Obesity)-Europe, RIO-North America, RIO-Lipids, and RIO-Diabetes (64). Although rimonabant (initially marketed as Acomplia® in Europe) produced weight loss and significant improvement in waist circumference, HDL cholesterol, insulin resistance, triglycerides, and adiponectin, nonetheless it has been recently withdrawn from the worldwide market because of increased rates of depression, anxiety, and suicide among patients who received the drug. Another clear example of the potential adverse effects of the chronic use of rimonabant is human fertility, where endocannabinoid signaling through CB$_1$R is crucial for oviductal transport, implantation, and development of embryos, as well as for sperm motility and acrosome reaction (97). Many other CB$_1$R antagonists are under clinical investigation; they are not only seen as antiobesity drugs, but also as therapeutics for other pathologies, including neurodegenerative diseases and nicotine or alcohol dependence.

SUMMARY POINTS

1. Endocannabinoids are lipid signals that exert manifold actions in the CNS and peripheral tissues by binding to different receptors (CB$_1$R, CB$_2$R, CB$_3$R, TRPV1) and thus triggering different signaling pathways.

2. The biological activity of endocannabinoids is subjected to a metabolic control, i.e., synthetic and hydrolytic enzymes regulate the intracellular concentration of these substances and hence their effects.

3. Exogenous and endogenous cannabinoids are present in food items, in particular in milk where they may provide a stimulus to the pup for suckling. Dietary unsaturated fatty acids and fish, olive, or safflower oils can influence brain endocannabinoid levels, as does a ketogenic diet.
4. The endocannabinoid system controls food intake and energy balance through multiple central and peripheral mechanisms, including synthesis of catabolic (proopiomelanocortin, CART, corticotropin-releasing hormone) and anabolic (neuropeptide Y, agouti-related protein, melanin-concentrating hormone) proteins in the hippocampus, and fatty acid and triglyceride biosynthesis in adipocytes and hepatocytes. Endocannabinoids are also involved in glucose tolerance, and a hyperactive endocannabinoid system is associated with obesity.

5. A dysregulated endocannabinoid signaling is heavily involved in eating disorders, cardiovascular diseases, and gastrointestinal pathologies, suggesting that endocannabinoid-oriented drugs might be next-generation therapeutics to treat these conditions in humans.

FUTURE ISSUES

1. Will drug designers develop novel compounds with improved selectivity toward distinct elements of the endocannabinoid system?

2. Will therapeutics unable to cross the blood-brain barrier become more effective than drugs acting both centrally and peripherally?

3. To what extent can we tolerate a chronic administration of ECS-oriented drugs, like the CB₁ receptor antagonist SR141716A (rimonabant or Acomplia®)?

4. How many as-yet-unknown ECS elements are to be discovered, and how could they contribute to the effect of the “selective” inhibitors or antagonists developed so far?

5. Is it possible that using an ECS-targeted pill will result in long-term healthy weight loss?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Figure 2

Biological functions of endocannabinoids in the central nervous system and peripheral tissues.
Figure 3
Central and peripheral effects of CB\(_1\)R activation on food intake and energy metabolism. FA, fatty acid; TGs, triglycerides.

Figure 4
Effects of CB\(_1\)R and CB\(_2\)R activation on cardiovascular pathologies.
Figure 5
Site of action of URB597 and rimonabant. eCBs, endocannabinoids; EtNH$_2$, ethanolamine; AA, arachidonic acid; AEA, anandamide; FAAH, fatty acid amide hydrolase.
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