Essays in Biochemistry (2020) EBC20190086
https://doi.org/10.1042/EBC20190086

PORTLAND PRESS

Review Article

The endocannabinoid system

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Thirty years ago, the discovery of a cannabinoid (CB) receptor that interacts with the psychoactive compound in *Cannabis* led to the identification of anandamide, an endogenous receptor ligand or endocannabinoid. Research on endocannabinoids has since exploded, and additional receptors along with their lipid mediators and signaling pathways continue to be revealed. Specifically, in humans, the release of endocannabinoids from membrane lipids occurs on demand and the signaling process is rapidly attenuated by the breakdown of the ligand suggesting a tight regulation of the endocannabinoid system (ECS). Additionally, the varying distribution of CB receptors between the central nervous system and other tissues allows for the ECS to participate in a wide range of cognitive and physiological processes. Select plant-derived 'phyto'cannabinoids such as Δ-9-tetrahydrocannabinol (Δ^9-THC) bind to the CB receptors and trigger the ECS, and in the case of Δ^9-THC, while it has therapeutic value, can also produce detrimental effects. Current research is aimed at the identification of additional phytocannabinoids with minimal psychotropic effects with potential for therapeutic development. Although decades of research on the ECS and its components have expanded our understanding of the mechanisms and implications of endocannabinoid signaling in mammals, it continues to evolve. Here, we provide a brief overview of the ECS and its overlap with other related lipid-mediated signaling pathways.

Introduction and historical context

Endocannabinoids are lipids that are produced endogenously in the human body and bind to and activate cannabinoid (CB) receptors. They include the arachidonic acid-containing metabolites, *N*-arachidonylethanolamine (AEA or anandamide) and 2-arachidonylglycerol (2-AG). These two endocannabinoids are derived from different metabolic pathways [1], and both belong to families of related lipid species—namely the *N*-acylethanolamines (NAEs) and monoacylglycerols (MAGs), most of which are CB receptor-inactive [2,3]. CB receptors take their name from their ligand Δ-9-tetrahydrocannabinol (Δ^9-THC), which is a psychoactive terpenoid produced in *Cannabis sativa*. Interestingly, neither anandamide nor 2-AG show marked structural similarity to Δ^9-THC despite their action as agonists on the same receptor family (Figure 1) [4,5]. Genes encoding CB receptors were identified in the 1990s [4,5], and the endocannabinoids, anandamide and 2-AG, were discovered shortly thereafter [6]. In the last two decades, details have emerged on the machinery for endocannabinoid signaling and the number of physiological and behavioral processes that are regulated by this signaling pathway have expanded remarkably. In fact, this general lipid signaling pathway appears to have aspects that are conserved evolutionarily well beyond vertebrates [7–9].

Received: 27 May 2020
Revised: 26 June 2020
Accepted: 26 June 2020

Version of Record published:
10 July 2020

Cannabinoids

The term CBs originally referred to the natural terpenoids or phenolic compounds that were identified in *Cannabis sativa*. Major CBs are derived as the product of decarboxylation of their corresponding 2-carboxylic acids, such as Δ^9-THC from Δ^9-tetrahydrocannabinolic acid A. More than 100 such compounds were identified in *C. sativa* and later in other plants, which are together referred

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https://doi.org/10.1042/EBC20190086

(A)

Cannabinoids	Category	Select Compounds
Phytocannabinoids	Psychoactive	Δ^9-Tetrahydrocannabinol Δ^9-Tetrahydrocannabivarin Cannabinol
	Non-psychoactive	Cannabidiol Cannabidivarin Cannabigerol Cannabichromene
Endocannabinoids	Major	Anandamide 2-arachidonyl glycerol
	Minor	Noladin ether Virodhamine *N*-Arachidonyl dopamine Oleamide
Cannabimimetics	*N*-Acylethanolamines	*N*-Palmitoylethanolamine *N*-Oleoylethanolamine *N*-Stearoylethanolamine
	Terpenoids & Phenolics	β-Caryophyllene Pristimerin Salvinorin A

(B)

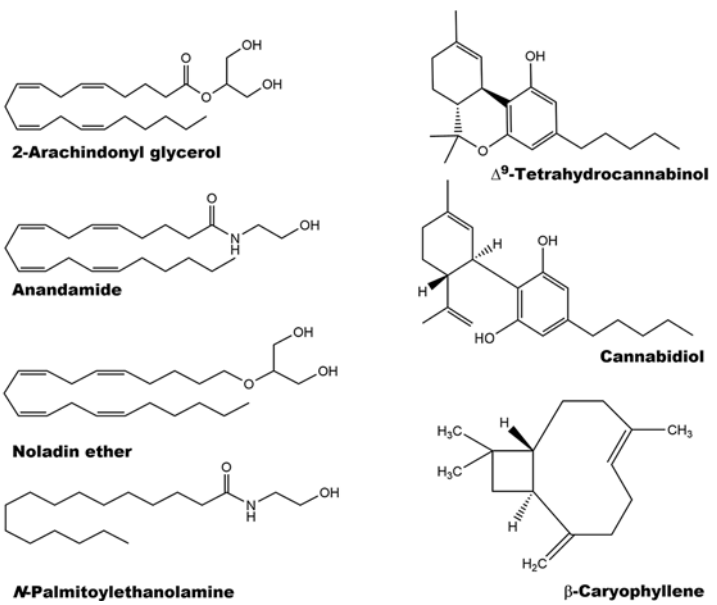

Figure 1. Select groups and examples of phytocannabinoid, endocannabinoid and cannabimimetic metabolites (A) with structural comparisons of representative examples (B).

Anandamide and 2-AG are the principal endogenous ligands that define the classical endocannabinoid signaling system (ECS).

to as phytocannabinoids [10]. Among these, Δ^9-THC is the most well-known psychoactive CB that has the ability to reduce pain perception and is also neuroprotective. The effects of Δ^9-THC on human brain are attributed to its ability to interact with G-protein coupled receptors (GPCR), and initiate a cascade of signaling mechanisms. There are two such major CB-binding receptors, CB1 and CB2, in addition to other candidate CB receptors toward which Δ^9-THC serves as a partial agonist with similar affinity [11,12]. Subsequent to the characterization of these receptors in mammals, endogenous ligands or endocannabinoids such as anandamide and 2-AG also were discovered [6,13,14].

Thus, the current CB term is expanded to include not only phytocannabinoids but also endocannabinoids and synthetic CBs that are able to interact with the CB receptors and modulate the endocannabinoid system (ECS; Figure 1). Additionally, certain phytochemicals that are structurally distinct from phytocannabinoids also can act as agonists or antagonists of CB receptors or interact with ECS. For example, β-caryophyllene [15] mimics CBs by functioning as a CB2 agonist while salvinorin A, although a selective κ-opioid agonist that does not directly interact with CB receptors, exhibits some of its effects through cross-talk between opioid and CB receptors [16–18]. Such compounds with an ability to functionally mimic classical CBs but are derived from plants beyond *Cannabis* are referred to as cannabimimetic compounds [19,20]. Highlighting the importance of homeostatic regulation of the ECS, the role of various CBs and the ECS in neurological disorders was discussed extensively in a recent review [21], although the ECS operates in many tissue systems outside the central nervous system.

Phytocannabinoids

An increased interest in pharmacological properties of CBs led to discovery of diverse array of phytocannabinoids that differ in their degree of psychoactivity (Figure 1). The classical CBs, psychotropic or non-psychotropic are mostly the oxidative or decarboxylated derivatives of cannabigerolic acid [22]. In addition to Δ^9-THC, the psychoactive group includes the subclass tetrahydrocannabivarin and cannabinol. The non-psychoactive CBs are grouped as cannabidiols (CBDs), cannabigerols and cannabichromenes. Several detailed reviews on the topic of phytocannabinoids and their functional activities in humans have emerged, including a systematic review of the phytocannabinoid inventory [10], molecular pharmacology [23], targets [24], physiological implications [25], and discussion of cannabimimetic ligands, beyond the *Cannabis*. More recently, the non-psychoactive CBD also has drawn considerable attention for its antioxidative and anti-inflammatory properties [26], effects on obesity [27] and cancer [28] and for the treatment of psychosis [29], epilepsy [30,31] and other neurological disorders [32]. Ongoing searches for additional phytocannabinoids with higher specificity and therapeutic value are constantly updating the list with newly discovered, bioactive compounds. Added to the list are the recently discovered Δ^9-tetrahydrocannabiphorol (Δ^9-THCP; [33]) with higher *in vivo* cannabimimetic activity than Δ^9-THC, and a butyl homolog of Δ^9-THC, Δ^9-tetrahydrocannabutol with comparable affinity to CB receptors as Δ^9-THC [34] from *C. sativa*. There are a multitude of signaling pathways by which these phytocannabinoids could potentially interact with endocannabinoids, eicosanoids and associated receptors in humans to exert their therapeutic effects. Identification of such phytocannabinoids and enhanced characterization of their signaling activities continues to expand and improve the utilization and regulation of medicinal *Cannabis*.

Endocannabinoids

Anandamide, the first discovered endogenous ligand of a CB receptor, in which *ananda* refers to bliss in Sanskrit, was identified in porcine brain extracts in 1992 [6]. Identification of an endogenous ligand that had the characteristic effect of psychotropic cannabinoids provided biological relevance for both the CB receptors and this obscure class of lipids. Anandamide or arachidonylethanolamide is an ethanolamide derivative of arachidonic acid and a member of a larger class of NAE lipids, and thus also referred to as NAE 20:4. Subsequently, *N*-homo-γ-linolenoylethanolamine (NAE 20:3(n-6)) and *N*- docosatetraenoylethanolamine (NAE 22:4(n-6)), that are CB-receptor active ethanolamide derivatives of long-chain polyunsaturated fatty acids, also were isolated from porcine brain [35]. However, it was the isolation of 2-AG from canine intestines [13,14], a more abundant lipid, relative to the other known endocannabinoid ligands and an efficacious agonist of both CB1 and CB2 receptors than anandamide and Δ^9-THC [36–40] that changed the landscape of endocannabinoid signaling. The concentration of 2-AG is approximately 170-times greater than that of anandamide and acts as a full and effective agonist of both CB1 and CB2 receptors [41]. The list of endocannabinoids was further expanded by additionally discovered ligands such as 2-AG ether (2-AGE or noladin ether; [42]), capsaicin-like *N*-arachidonoyl dopamine (NADA; [43,44]) and *O*-arachidonoyl ethanolamine (O-AEA or virodhamine [45]), which are considered to play more specific roles in modulation of cannabinoid neurophysiology via parallel or overlapping signaling pathways (Figure 1).

The ECS components

The CB receptors, their ligands and the metabolic enzymes that are responsible for their synthesis and degradation are the integral parts of the ECS. The most well-studied components of the ECS in mammalian systems are the two CB receptors and their major endocannabinoid ligands, anandamide and 2-AG. Several enzymes involved in the synthesis and degradation of anandamide and 2-AG also received prominent attention due to their ability to modulate endocannabinoid concentration and the resulting signaling response.

Essays in Biochemistry (2020) EBC20190086
https://doi.org/10.1042/EBC20190086

Receptors and ligands

The two classical receptors of ECS, CB1 [4] and CB2 [5] are GPCR proteins that were originally discovered in the membranes of neural cells and macrophages, respectively. The discovery of these CB receptors was prompted by the desire to understand the long-standing medicinal role of *Cannabis* extracts as well as its euphoric effects in humans. As members of the GPCR family, both CB receptors contain seven transmembrane spanning domains, share 44% similarity between their two amino acid sequences and are generally coupled to the activation of heterotrimeric G proteins of the G_i and G_o classes [11,46]. Among the two receptors, CB1 is predominantly expressed in brain and neural cells, affecting the functions and responses mediated through central nervous system. Interestingly, an alternate splice form CB1 with a deletion of 33 amino acids in the N-terminus is expressed in liver, pancreas and adipose tissues. This CB1 isoform is involved in glucose metabolism and antagonists to this receptor increased weight loss and reduced obesity [47]. In the case of CB2, its predominant isoform is expressed in T cells of the immune tissues and the peripheral nerve endings but absent from the brain, while an alternate isoform was found in the reward center of brain, as well as in testes [48]. Among the two endocannabinoids, anandamide is a partial agonist with higher ability to bind to CB1 receptors but with negligible affinity towards CB2, relative to 2-AG. In contrast, 2-AG is full agonist with low to moderate affinity toward both CB1 and CB2 [39,40]. With much higher ability to evoke functional response than anandamide, the efficacy of 2-AG in mediating CB receptor-dependent G-protein activity is higher and thus is considered as a robust and natural ligand for CB receptors [39].

The functional and metabolic plasticity of endocannabinoids allows them to regulate a wide range of responses, directly and indirectly, through other molecular targets (Table 1). Both 2-AG and anandamide, like the major phytocannabinoids, Δ^9-THC and CBD show a degree of promiscuity by interacting with other receptors beyond CB1 and CB2. As such, the occurrence of certain additional and also orphan receptors capable of potentiating ECS was long suspected, which was further confirmed by the presence of the CB responsiveness even in CB receptor knockout mutants [21]. For example, both anandamide and 2-AG can activate other targets such as the transient receptor potential of vanilloid (compounds with vanillyl group such as capsaicin) type-1 (TRPV1) channels or ionotropic endocannabinoid receptors [49] to inhibit Ca^{2+} channels and also affect TRP subfamily M member 8 (TRPM8). Like anandamide, 2-AG can also interact with TRPV1 and additionally γ -aminobutyric acid (GABA) receptors [14,21]. The TRPV1 channels are also activated by *N*-acyl amides, *N*-palmitoylglycine (PalGly) and NADA [50–52]. Similarly, endocannabinoids act as agonists for nuclear receptors, such as the peroxisome proliferator-activated receptor α (PPARα) to play a role in metabolic and anti-inflammatory and neuroprotective responses (Table 1). The α, β/δ and γ subtypes of PPAR family are also activated to varying degrees by eicosanoid derivatives such as 15-hydroxyeicosatetraenoic acid (15-HETE) [53].

Among the orphan GPCRs, GPR18, GPR55 and GPR119 not only share sequence homology with CB receptors but also display overlapping roles (Table 1) [54]. Of these, GPR18 has been proposed as a candidate CB receptor with *N*-arachidonoyl glycine (NAraGly) as an endogenous agonist; other agonists include AEA, 2-AG and *N*-acyl glycines and ethanolamines [55]. The activation of GPR18 in a pertussis toxin (PTX)-sensitive manner by its agonists produces antinociceptive and anti-inflammatory effects. On the other hand, lysophosphatidylinositol (LPI) showed high affinity for GPR55 [56] and lysophosphatidic acid (LPA) for GPR92 [57] but these agonists do not bind to CB receptors *per se*. In fact, GPR92 also interacts with NAraGly but is now considered as a newly identified $G_{12/13}$ and G_q-coupled LPA_5 receptor that induces cyclic adenosine monophosphate (cAMP) formation upon activation and is largely co-localized with TRPV1 [58].

Flexibility to interact with receptors beyond CB receptors is also a feature of other minor endocannabinoids such as noladin ether, NADA and virodhamine (Table 1) [42,43,45]. Noladin ether, which is metabolically more stable than 2-AG is an agonist of both CB1 and CB2 receptors. Additionally, noladin ether also is a partial agonist of TRPV1 and PPARα, and serves a neuroprotective role among other functions [59,60]. Interestingly, NADA is a CB1 and TRPV1 agonist but does not interact with dopamine receptors, and because of its high efficacy, NADA was suggested as putative ligand for TRPV1 [43,44]. Virodhamine, which is an arachidonic acid and ethanolamine joined by an ester linkage has partial agonist/antagonist properties at the CB1 and full agonist at CB2 receptor [45]. Together with anandamide, virodhamine also modulates the activity of GPR55 affecting both inflammatory and neuropathic pain [56,61].

Another long-chain acylamide, commendamide (*N*-acyl-3-hydroxy palmitoyl glycine), which shares similarity to mammalian endocannabinoids, is a GPCR G2A/132 agonist [62]. Commendamide was identified as a natural product in the culture exudate of *Bacteroides vulgatus*, a commensal bacterium and it can activate GPCR G2A/132 at an order of magnitude lower concentration than by NF-κB. The GPCR G2A/132 has been since implicated in host–microbial interactions and is thought to play a role in autoimmune disease and atherosclerosis [63]. Additionally, oxidative

Table 1 CB and several other receptors, principal ligands, ligand activity and representative functions

Receptors	Ligands	Activity	Select functions	References
CB receptors				
CB1/CB2	AEA	Partial agonist	Regulation of mood perception, cognition, fear, memory, food intake, visual development and locomotion	[11,21,40]
CB1/CB2	2-AG	Agonist	Inhibitory retrograde neuromodulators	
CB1/CB2	Noladin ether	Agonist	Neuroprotective by activation of PPARα; increases GABA uptake	[42,76,112]
CB1	NADA	Agonist	Antioxidant, neuroprotectant	[43,44]
CB1	Virodhamine	Partial agonist or antagonist	Lowers body temperature, modulate neurotransmitter release	[45]
CB2	Virodhamine	Agonists	Not understood	[45]
Transient receptor potential (TRP) channels				
TRPV1/VR1	AEA, 2-AG, PEA, NADA, OLDA, NGABA, NGly/Asp/Ser Noladin ether, 2-AG	Agonists/partial agonists	Nociception, vasodilator action, thermal hyperalgesia	[43,113,114]
TRPV2	NPro, NTyr, AEA, 2-AG, NADA	Agonist	Effects inflammatory and chronic pain	[50]
TRPV3	NVal	Antagonist	Perception of pain and itch	[50]
TRPV4	2-AG, AEA, Oxidative metabolites of AA, NTrp, NTyr	Agonists	Regulation of systemic osmotic pressure in the brain, and plays a role in vascular function, skin barrier function and nociception	[115]
TRPA1	AEA, AA	Agonists	Activation of peripheral sensory neurons, nociception	[116–118]
TRPM8	AEA, NADA, Virodhamine	Antagonists	Thermal sensor, role in cancer	[119]
Nuclear receptors				
PPAR-α	AEA (?), PEA, OEA and N-oleoyl glycine, Oxidative products of 2-AG (15-HETE-G), Noladin ether, Virodhamine	Agonists	Anti-inflammatory and neuroprotective; reduces food intake lipid sensor, peroxisome proliferator	[21,120]
PPAR-γ	AEA, 2-AG oxidative products of AEA (15-HETE, 9/13-HODE)	Agonists	Fatty acid metabolism, anti-inflammatory	[21,120]
Orphan GPCRs				
GPR18	N-arachidonylglycine, AEA, 2-AG	Agonists	Regulates metabolic disorders, antinociceptive and anti-inflammatory effects	[122–124]
GPR G2A/132	9-HODE, other oxidative products of LA, AA, commendamide	Agonists	Atherosclerosis, inflammation	[64]
GPR55 (LPI Receptor)	LPI, AEA, 2-AG and Virodhamine, PEA, Noladin ether	Agonists/partial agonists	Effects balance between pro- and anti-inflammatory effects, neuropathic pain	[61,121,124]
GPR92 (LPA5 Receptor)	LPA, NAraGly (NAG)	Agonists	Development and stem cell functions, sensory nervous system	[57,58]
GPR110	DEA	Agonist	Neurodevelopmental control	[125]
GPR119	2-Acylglycerols, Oleamide, OEA, AEA, PEA	Agonist	Fat sensor, energy balance	[21,64]
Additional receptors				
GABAA	2-AG	Allosteric activator	Locomotion and sedation	[126]
Cav3.2 (T- type) Ca^{2+} channel	AEA, AA, long-chain fatty acid amides	Antagonists	Analgesia, neuronal and cardiac excitability, control in pace-making activity, role in pain, epilepsy, Parkinson's disease, and several forms of cancer	[127–129]

Note that these diverse receptors have overlapping endocannabinoid metabolites that serve as endogenous ligands.

Essays in Biochemistry (2020) EBC20190086
https://doi.org/10.1042/EBC20190086

metabolites of long-chain fatty acids, 9-hydroxyoctadecadienoic acid and 11-hydroxy-5,8,12,14-eicosatetraenoic acid are also potential ligands of G2A/132 [64], suggesting that G2A/132 mediates the action of internal and external lipid mediators. Consequently, many acyl amides act in a similar manner to the well-characterized endocannabinoids, anandamide and 2-AG, highlighting the complexity of physiological and behavioral regulation by the ECS and related, often overlapping, signaling networks.

Other endogenous fatty acid derivatives such as the acylglycerols, acylethanolamides, acyldopamides, primary acylamides and *N*-acylated amino acids also interact with non-CB receptors such as PPARα and TRPV1 [65], and some of the responses resemble ECS signaling. Bradshaw and Walker [66] have provided an excellent summary on the bioactive roles of these compounds. For example, PPARα and GPR119 are activated by the monounsaturated acylethanolamide, *N*-oleoylethanolamide (OEA) to regulate feeding behavior and body weight [67]. In fact, OEA also was recently identified as an endogenous ligand for GPR119 [68], which is now an attractive target for the treatment of obesity and related metabolic disorders. In addition to OEA, PPARα is also activated by leukotriene B4 [69] and by the most abundant type of NAE in mammals, *N*-palmitoylethanolamine (PEA). The anti-inflammatory and neuroprotective properties of endogenous PEA are mediated through PPARα [70]. More recently, like anandamide and 2-AG [56], PEA was shown to also activate GPR55 and GPR119. To this extent, PEA acts in synergy with anandamide and reduces the detection of pain stimulus (nociception; reviewed in [65]. By contrast, oleamide (a primary acylamide) acts antagonistically to inhibit anandamide uptake and/or degradation (reviewed in [71]). Overall, these non-CB-lipid mediators could demonstrate cannabimimetic effects by indirect enhancement of CB activity, referred to as entourage effects [72]. While it is clear that there are connections between CB receptor-inactive lipids and endocannabinoids, there is much to be learned regarding the interplay of various fatty acid lipid mediators with the ECS.

Metabolic enzymes

Endocannabinoid levels are maintained by their synthesis, typically on demand, and rapid degradation. Metabolism of endocannabinoids is an important feature of the ECSse their synthesis or degradation can have profound cellular and physiological consequences. The metabolic pathways for anandamide and 2-AG are quite distinct, although both are eicosanoid derivatives with structural similarities (Figures 2 and 3).

Anandamide and other NAEs can be generated by more than one pathway, depending on the physiological conditions and tissue type (Figure 2) [73]. The first and most prevalent pathway to be identified involves hydrolysis of a minor membrane lipid, *N*-arachidonylphosphatidylethanolamine (NArPE) by a *N*-acyl phosphatidylethanolamine (NAPE)-specific phospholipase D-like hydrolase (NAPE-PLD) [74]. Anandamide also may be generated by hydrolysis of NArPE by a phospholipase C (PLC) to phosphoanadamide, which is then acted on by phosphatases (PTPN22) to remove inorganic phosphate and release anandamide [75]. Additional pathways to form anandamide include generation of lyso-NArPE as an intermediate from NArPE by a group of IB secretory phospholipase A2 or by α/β domain-containing hydrolase 4 (ABDH4). Lyso-NArPE is then either metabolized to anandamide by a calcium-independent lyso-phospholipase D (PLD) [76], or to glycerophospho-*N*-arachidonoylethanolamine (GpAEA) by a second deacylation catalyzed by ABDH4, respectively. GpAEA is then acted on by a metal-dependent, glycerophosphodiesterase (GDE1) to generate anandamide [77]. Lyso-NArPE can also contribute to synthesis of phosphoanandamide through the action of a lyso-PLC [75]. Interestingly, the fatty acid amide hydrolase (FAAH) that is well known for anandamide hydrolysis was shown to be capable of anandamide formation under high concentrations of arachidonic acid and ethanolamine [78,79].

The formation of 2-AG is by a different series of reactions and from a different membrane lipid precursor (Figure 3). 2-AG formation involves first the generation of diacylglycerol (DAG) as an intermediate from phosphatidylinositol 4,5-bisphosphate (PI4,5P), which is catalyzed by PLCβ [80]. DAG lipases (DAGLs) (either α or β isoforms) subsequently convert DAG into 2-AG [80]. Alternatively, formation of 2-AG also can proceed by the conversion of PI lipids into 2-arachidonoyl-lyso PI, by the action of a phospholipase A1 (PLA1) followed by lyso-PLC [81]. Hydrolysis of 2AG-LPA by an LPA phosphatase also has been shown to generate 2-AG [82].

The inactivation of endocannabinoids features their degradation by distinct hydrolytic enzymes. Anandamide and other NAEs are hydrolyzed by FAAH to non-esterified (free) fatty and ethanolamine, and the mechanism of this amidase enzyme has been well characterized [83–85]. By contrast, 2-AG is degraded to arachidonic acid (AA) and glycerol, primarily by MAG lipase (MAGL), but other serine hydrolases such as FAAH [86], ABHD6 and ABHD12 also can hydrolyze 2-AG [87]. Additionally, *in vitro* studies have shown the potential for neuropathy target esterase [88] and hormone sensitive lipase [89] to hydrolyze MAGs including 2-AG; however, their relevance to 2-AG hydrolysis in a biological context is not well understood. Because the levels of these endocannabinoid ligands determine

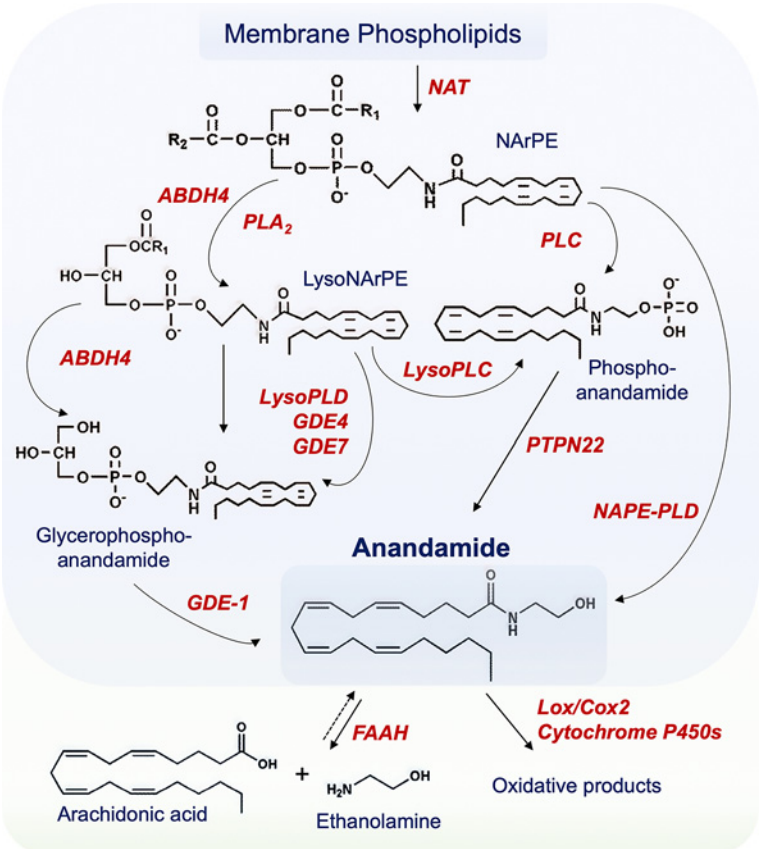

Figure 2. Schematic of anandamide metabolic pathway

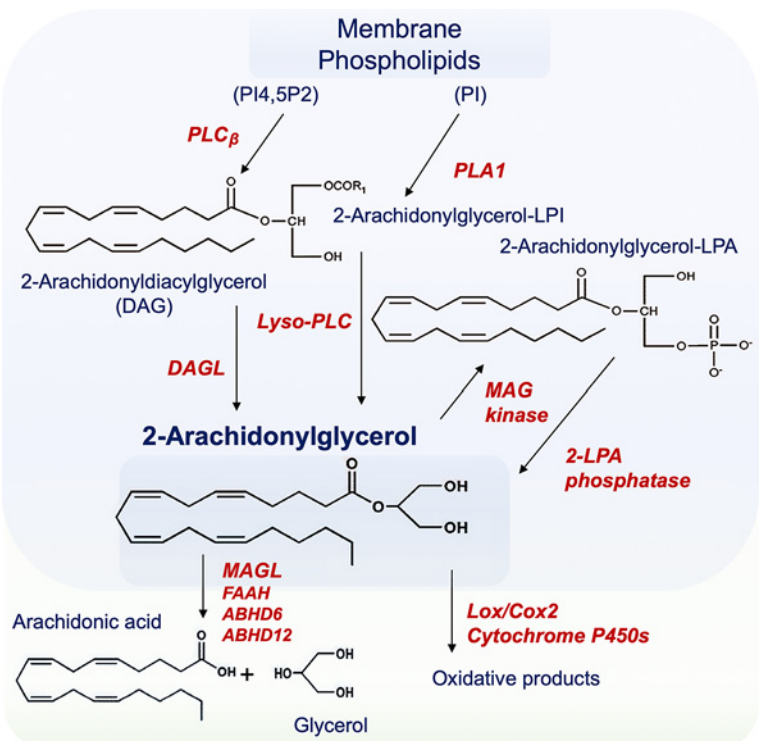

Figure 3. Schematic of 2-AG metabolic pathway

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https://doi.org/10.1042/EBC20190086

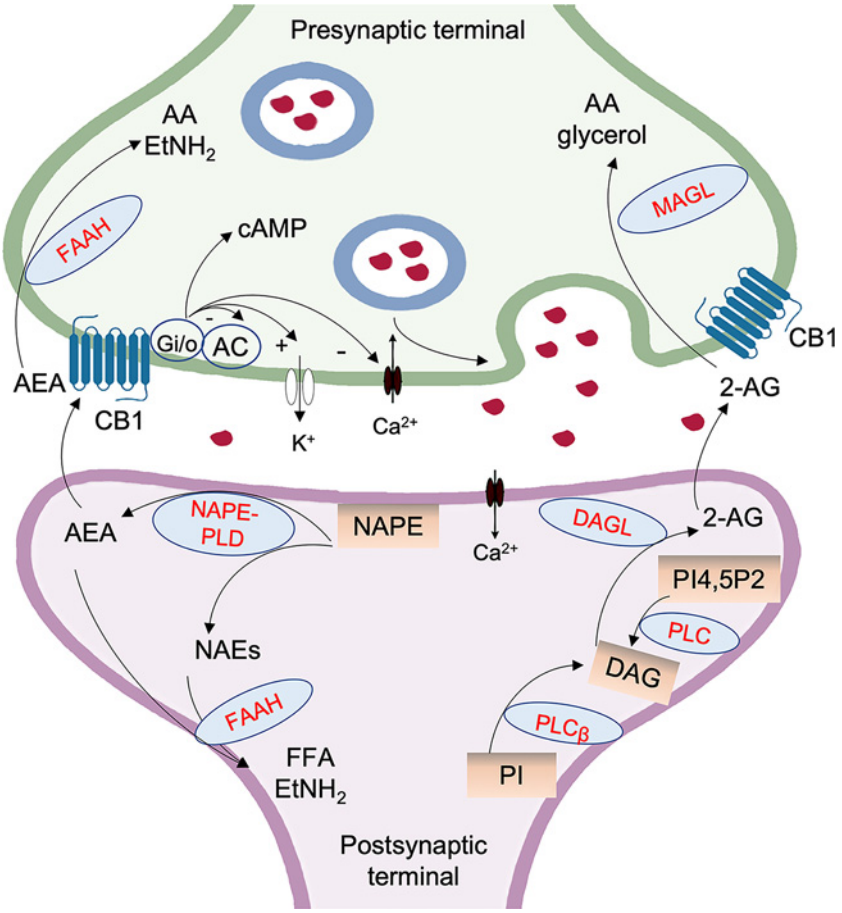

Figure 4. Schematic example of retrograde signaling mediated by the ECS

Anandamide or 2-AG is formed on demand in postsynaptic plasma membranes by lipases (NAPE-PLD or DAGL, respectively), and both signaling molecules traverse the synaptic cleft to interact with CB1 receptors on presynaptic membranes. These activated CB1 receptors in turn activate heterotrimetric G proteins to inhibit neurotransmitter (red) secretion through the modulation of membrane-bound ion channels. Consequently, these endocannabinoids produced in postsynaptic cells can modulate neuronal signal propagation from presynaptic neurons. The endocannabinoids are inactivated by hydrolysis by FAAH or MAGL in presynaptic cells. See text for details. Abbreviations: PIP2, phosphatidylinositol 4,5 bisphosphate; Gi/o, heterotrimeric G protein (inhibitory/olfactory).

the signaling activity of the ECS, and the ECS dysfunction is manifested in numerous pathologies, both FAAH and MAGL are potential targets for multiple therapeutic interventions [90].

Additionally, long-chain polyunsaturated NAEs such as anandamide, and 2-AG are also oxidized by fatty acid oxygenases such as lipoxygenases, cyclooxygenases and cytochrome P450s [53,91]. Their diverse oxidative metabolites, analogous to the oxidative products of arachidonic acid, have significant physiological activities as they interact with various other signaling pathways as well as ECS signaling [92,93]. For example, a P450-derived epoxide of anandamide is a potent agonist for CB2 [91]. Oxidation of 2-AG by cyclooxygenase-2 (COX-2) produces prostaglandin glycerol esters [94,95] that act as an agonist for purinoceptor 6 (P2Y6), a uridine diphosphate (UDP) receptor [96]. Lipooxygenase (LOXs) generate hydroperoxy derivatives of anandamide and 2-AG that are involved in eicosanoid signaling [97,98]. At last, 2-AG is also phosphorylated by acyl glycerol kinase(s) to produce LPA, which is an activator of various signaling pathways [99].

Together, these various parallel and redundant pathways involved in metabolism of the two major endocannabinoids and their interacting partner receptors are expected to provide plasticity as well as specificity in modulating diverse ECS responses. Such complex network of interactions between the ligands, receptors and other metabolites that are directly or indirectly involved with ECS are referred to as endocannabinoidome, a term coined by DiMarzo [21], and require future investigation.

Activation of the ECS

Endocannabinoid signaling has been implicated in a number of physiological and cognitive processes in humans. The endocannabinoids are well-known modulators of various neurological functions such as perception of pain, mood, memory, sleep, appetite etc. [21,100]. The ECS also plays an important role in activation of immune system, pre- and postnatal development, embryo implantation and fertility, among others [101,102]. Thus, maintaining physiological homeostasis is considered as the primary function of endocannabinoid signaling. The biological consequences of endocannabinoids result from their interactions with several target proteins, including CB receptors, G-proteins and other downstream effectors. As such, synthesis, receptor activation and degradation of endocannabinoids are all highly regulated and is believed to start 'on-demand,' via a stimulus-dependent synthesis. The formation of anandamide and 2-AG, from membrane lipid precursors is initiated by increase in intracellular Ca^{2+} and subsequent activation of the hydrolytic enzymes (reviewed in [103]). For example, endocannabinoid-mediated retrograde signaling starts with the synthesis of 2-AG or AEA in the post-synaptic terminal, in response to increased intracellular Ca^{2+} and/or activated G_q/11-coupled receptors (Figure 4). 2-AG or AEA is then released into the synaptic space and moves to bind to and activate CB1 receptor in the presynaptic terminal. Activated CB1 receptor can suppress the release of neurotransmitter either by inhibiting voltage-gated Ca^{2+} channels to reduce presynaptic Ca^{2+} influx (short-term plasticity) or adenylyl cyclase-mediated cAMP/protein kinase A (PKA) pathway (long-term plasticity) [104,105]. The signaling activity is attenuated or inhibited by the degradation of 2-AG by MAGL and AEA by FAAH expressed in synaptic terminals and glial cells [106]. Anandamide also serves as a full or partial agonist of TRPV1 depending on its concentration and tissue selectivity to induce Ca^{2+} response, and also facilitate long-term depression by regulating post-synaptic function [107–110].

Beyond the neural system, CB1-dependent signaling pathways influence $G_{i/o}$-dependent inhibition of adenylyl cyclase and voltage-gated Ca^{2+} channels and activation of K^+ currents and nitric oxide signaling in the tissues of liver, heart,productive organs and bone. In this context, CB1 receptors interact with GPCR kinases, β-arrestins, GPCR-associated sorting proteins and other GPCRs for heterodimerization. CB1 also interacts with factors associated with neutral sphingomyelinase and the novel CB receptor-interacting proteins, $CRIP_{1a/b}$. Overall, these interactions regulate intracellular trafficking, desensitization, down-regulation, signal transduction and constitutive activity of CB1 receptors [46,111]. The intracellular CB2-dependent pathway also inhibits adenylyl cyclase, stimulates mitogen-activated protein kinase and activates phosphoinositide 3-kinase pathways and *de novo* ceramide production or COX-2 induction. CB2 also can interact with some of the proteins similar to those of CB1 receptors, such as β-arrestins [11] for desensitization. In essence, endocannabinoids are versatile modulators of ECS, which act through a variety of receptor-triggered cellular responses as part of a finely tuned system.

Summary and conclusions

- The ECS has come to be defined as the two principal endogenous ligands, their cognate receptors and the enzymes that regulate their internal concentrations.
- The ECS was originally discovered as the target of the psychotropic phytocannaboinoid, Δ^9-THC, produced in *Cannabis* flowers. However, in recent years it is clear that additional plant-derived, microbial-produced, and endogenous lipid mediators interact with the ECS and other receptor-mediated signaling pathways to regulate a range of complex cellular processes.
- Future research will continue to unravel the ECS interaction with partner pathways as a means to reveal the intricate network of lipid-mediated regulatory systems that determine a multitude of physiological and behavioral outcomes.
- Therapeutic intervention in the dysregulation of the ECS will no doubt involve new phytocannabinoids and various synthetic CBs with which to control an increasing list of ECS- related pathologies.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work in the authors' laboratories on lipid signaling and partial support for the preparation of this article has been supported by the National Science Foundation Division of Integrative Organismal Systems [grant numbers IOS-1456917 (to A.K.), IOS-1656263 (to K.D.C.)].

Author Contribution

Both authors contributed to the writing of this manuscript.

Acknowledgements

We thank Drs. Charlene Case, University of North Texas and Lihang Xie, East Tennessee State University for their excellent assistance with manuscript preparation.

Abbreviations

2-AG, 2-arachidonylglycerol; 2-AGE, 2-AG ether or noladin ether; ABDH, α/β domain-containing hydrolase; AEA, *N*-arachidonylethanolamine or anandamide; cAMP, cyclic adenosine monophosphate; CB, cannabinoid; CBD, cannabidiol; COX-2, cyclooxygenase-2; DAG, diacylglycerol; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GpAEA, glycerophospho-*N*-arachidonoylethanolamine; GPCR, G-protein coupled receptor; MAG, monoacylglycerol; MAGL, MAG lipase; NADA, *N*-arachidonoyl dopamine; NAE, *N*-acylethanolamine; NAPE, *N*-acyl phosphatidylethanolamine; NAraGly, *N*-arachidonoyl glycine; NArPE, *N*-arachidonylphosphatidylethanolamine; OEA, *N*-oleoylethanolamide; PEA, *N*-palmitoylethanolamine; PKA, protein kinase A; PLC, phospholipase C; PLD, phospholipase D; PPARα, peroxisome proliferator-activated receptor α; PTX, pertussis toxin; TRPV1, transient receptor potential of vanilloid type-1; UDP, uridine diphosphate; Δ9-THC, Δ-9-tetrahydrocannabinol.

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