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Opinion

Missing Pieces to the Endocannabinoid Puzzle

Mauro Maccarrone[1,2,*]

The most bioactive ingredient of cannabis (*Cannabis sativa* or *indica*) extracts, Δ^9-tetrahydrocannabinol (THC), was identified in the 1960s as one of more than 110 phytocannabinoids. It activates receptors of chemically different endogenous ligands (endocannabinoids) that, unlike THC, are metabolized by several enzymes of the endocannabinoid system. Here, the complexity of the plant-derived and endogenous cannabinoids (eCBs) is discussed, to better appreciate the challenge of: (i) dissecting their mutual interactions; (ii) understanding their impact on human pathophysiology; and (iii) exploiting them for human disease. To this aim, missing pieces to the eCB puzzle must be urgently found, by solving the 3D structures of key components, and interrogating noncanonical modes of regulation and trafficking of these lipid signals.

Plant-Derived and Endogenous Cannabinoids

It has been known for centuries that extracts from cannabis (*C. sativa* or *indica*) have been used to treat human diseases or for recreational purposes; yet, their bioactive agent was only identified in the 1960s as THC. Since then, more than 110 compounds have been isolated from the same plant, and are collectively known as cannabinoids or phytocannabinoids [1]. In addition, cannabis extracts contain other compounds (i.e., terpenoids, flavonoids, and sterols) that can modulate in various ways the activity of THC and congeners [1]. Many of these natural substances, and in particular cannabidiol, have stimulated intense research in recent years for their potential therapeutic efficacy for neurological disorders, alone or in combination with THC [2]. Yet, there is little understanding of the true chemical composition (and hence pharmacological efficacy) of a cannabis extract, because of changes that may depend on plant cultivars and growth conditions, as well as on extraction procedures and administration routes of ingredients. Of course, these uncertainties represent a warning for the proposed clinical indications of these natural compounds, summarized in Table 1 (see also Clinician's Corner). THC binds at and activates specific **G protein-coupled receptors** (see Glossary), known as **type-1 (CB_1)** and **type-2 (CB_2) cannabinoid receptors** [3–6] (Figure 1). The discovery of cellular targets of THC has triggered a quest for endogenous molecules able to activate them. Such endogenous ligands of CB_1 and CB_2 were identified in the 1990s as **anandamide (AEA)** [7] and 2-arachidonoylglycerol (2-AG) [8,9]; two of the most-active and best-studied eCBs [10,11]. Of note, THC, AEA, and 2-AG, although markedly different in their chemical structures, share three **pharmacophores** that interact with the same targets, and activate common **signaling pathways** [12]. The ability of the organism to metabolize eCBs (i.e., amides and esters of fatty acids), but not THC (i.e., a terpeno-phenol) appears also significant. Thus, the latter compound is not subjected to the stringent metabolic control that regulates eCBs, as discussed below in more detail.

Complexity of the eCB System

The eCB system (ECS) is composed of receptors, transporters, and enzymes that support and control the manifold actions of eCBs, both in the central nervous system [13,14] and at the periphery [10,11]. In particular, it is striking that at least 12 receptors are activated by eCBs in the same cell; both on the plasma membrane (where they can have an extracellular or an intracellular binding site), and in the nucleus. The eCB-binding receptors can largely be grouped into three functional families (Figure 1): (i) receptors with an extracellular binding site such as CB_1 and CB_2 [3,4], as well as G protein-coupled orphan receptors (GPRs) 55 [15] and 119 [16]; (ii) receptors with an intracellular binding site such as **transient receptor potential vanilloid 1 (TRPV1)** [17], TRPV2, TRPV3, TRPV4, TRPA1, and TRPM8 [18]; and (iii) transcription factors such as nuclear **peroxisome proliferator-activated receptors (PPARs)** α [19], γ [20], and δ [21]. Receptor-mediated activities of eCBs depend on their cellular concentration, that in turn depends on a balance between synthesis and degradation operated by different biosynthetic and hydrolytic enzymes. Calcium-dependent *N*-acyltransferase (NAT) [22], *N*-acyl phosphatidylethanolamines-specific phospholipase D (NAPE-PLD) [23],

Highlights

Cannabis (*C. sativa* or *indica*) extracts have emerged as complex mixtures of more than 110 compounds, termed (phyto)cannabinoids. The most active of them is THC, yet it is now apparent that other cannabinoids can also modulate THC effects and/or have their own biological activity.

THC activates specific receptors in the human body, known as type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors, that normally bind eCBs.

eCB signaling is complex, as it engages 12 receptors and many biosynthetic and degradative enzymes, as well as transporters and storage organelles.

Fine regulation of eCB content and receptor-mediated signaling is crucial, and it appears to occur via canonical and emerging noncanonical mechanisms.

THC is able to escape the stringent metabolic control to which the biological activity of eCB is subjected.

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Table 1. Proposed Clinical Indications of the Most Studied Phytocannabinoids

Phytocannabinoid	Proposed clinical indications[a]
THC	Antiemetic Treatment of post-traumatic stress disorder Treatment of sleep disorders Treatment of symptoms of dementia Appetite stimulant
Cannabidiol	Antipsychotic Treatment of symptoms of Parkinson's disease Anxiolytic Treatment of post-traumatic stress disorder Anti-inflammatory or antinociceptive effect Treatment of seizures
Cannabidivarin	Antiemetic Treatment of seizures
Cannabigerol	Appetite stimulant Treatment of Huntington's disease
Δ^9-tetrahydrocannabivarin	Anti-inflammatory or antinociceptive Treatment of seizures

[a]For more extended information see [2].

glycerophosphodiesterases (GDEs) 1, 4 ,and 7 [24], and α/β hydrolase domain containing protein 4 (ABHD4) [24] catalyze parallel routes for the release of AEA from phospholipid precursors; while fatty acid amide hydrolase (FAAH) [25] and *N*-acylethanolamine acid amidase (NAAA) [26] cleave AEA and other eCBs, terminating their signaling activity. Similarly, diacylglycerol lipases (DAGLs) α and β synthesize 2-AG [27], that instead is cleaved in several different ways by monoacylglycerol lipase (MAGL) [28], ABHD2 [29], ABHD6 [30], or ABHD12 [31]. In addition to synthesis and degradation, a further level of complexity in eCB metabolism is represented by the addition of oxygen to the fatty acid moieties of AEA and 2-AG by **cyclooxygenase-2 (COX-2), lipoxygenase (LOX) isozymes** such as 5-LOX, 12-LOX, and 15-LOX, and cytochrome P450; similar to the way these enzymes act on arachidonic acid [32]. The oxidative products of eCBs are endowed with their own biological activities, distinct from those of eCBs ([33], and references therein). Such a stringent metabolic control of eCB tone is further modulated by distinct transporters that facilitate the movement of eCBs both across the plasma membrane [34], and intracellularly [35], as well as by storage of eCBs in cytosolic organelles like adiposomes [36]. Altogether, these receptors, enzymes, and transporters form the ECS, as schematically depicted in Figure 1.

It should be appreciated that eCBs are short-lived (~15 min) substances, and that metabolic enzymes and transporters are responsible for their timely delivery (in the right concentration) to the right target in the cell. Therefore, a detailed knowledge of the 3D structures of all ECS elements, and hence of the molecular details of their interactions with exogenous (plant-derived) and endogenous signals, could turn the different ECS proteins into reliable, novel drug targets. The state of the art of 3D structures of ECS elements, and of their regulation through canonical and noncanonical mechanisms, is discussed below.

Structure of ECS Components

To date, 3D structures of 23 major components of the ECS have been resolved (Table 2). Of them, eleven are membrane-bound proteins (seven receptors and four enzymes); eight are cytosolic proteins (four enzymes and four transporters); three are nuclear transcription factors (PPARα, γ, and δ); and one enzyme (MAGL) is both membrane-bound and cytosolic. The 3D structures of the remaining

Glossary

Allosteric modulator: a compound that modulates enzyme or receptor activity by binding at an allosteric site other than the active site. Such allosteric binding causes a conformational change in the protein, that can be either positive (increased activity, due to PAMs) or negative (reduced activity, due to NAMs).
Anandamide (AEA): *N*-arachidonoylethanolamine, that is the amide of arachidonic acid [5,8,11,14-eicosatetraenoic acid, an essential (diet-derived) fatty acid] with ethanolamine, named after the Sanskrit word 'ananda' for inner bliss.
Biased signaling: due to biased ligands that are able to selectively trigger some signaling pathways, while avoiding (or even inactivating) others that are dependent on the same receptor.
Cannabinoid receptors (CBs): G protein-coupled receptors that bind THC, AEA, 2-AG, and other eCBs. Two subtypes are known as CB_1 and CB_2.
Cyclooxygenase-2 (COX-2): officially known as prostaglandin-endoperoxide synthase, is an oxygenase responsible for formation of prostanoids (like thromboxanes, prostaglandins, and prostacyclins) from arachidonic acid.
G proteins: family of guanine nucleotide-binding proteins that act as molecular switches between external stimuli and intracellular signals of a cell.
G protein-coupled receptors: seven-transmembrane domain receptors (also known as serpentine receptors), are a large family of proteins that detect molecules outside the cell, thus activating internal signal transduction pathways and cellular responses via G proteins. Of note, CB_1 is the most abundant member of this family in mammalian brain. When these receptors are orphan (i.e., their ligands are unknown), they are usually given the name GPR followed by a number, for example GPR55 and GPR119.
Isozymes (or isoenzymes): enzymes that catalyze similar reactions, but slightly differ from each other in chemical structure, and therefore kinetic properties.

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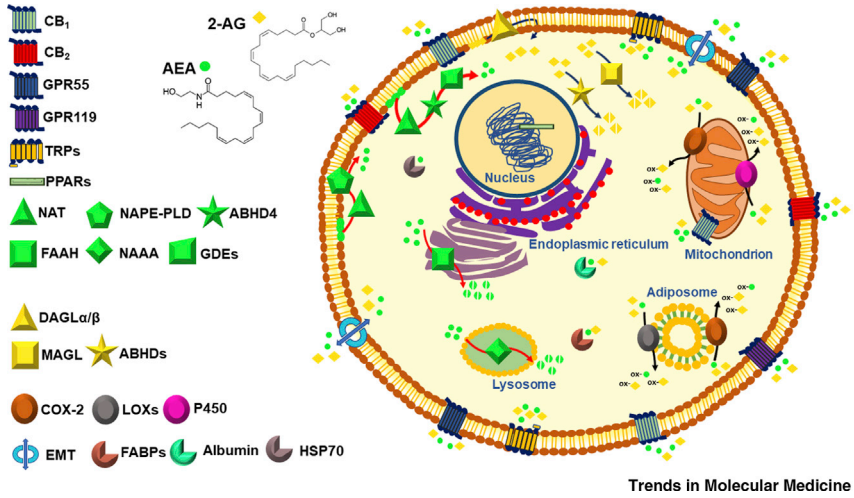

Figure 1. Overall View of the Endocannabinoid System.

Endocannabinoids can signal through various receptors on the plasma membrane (CB_1, CB_2, GPR55, GPR119, and TRPs) and in the nucleus (PPARs), and their biological activity is controlled by metabolic enzymes (NAT, NAPE-PLD, ABHD4, FAAH, and NAAA for the synthesis and degradation of AEA; DAGLα/β, MAGL, and ABHDs for the synthesis and degradation of 2-AG); by transport mechanisms across the plasma membrane (via a putative EMT); intracellularly (via FABPs, albumin, and HSP70); and by accumulation and storage in intracellular organelles called adiposomes. In the latter organelles, they can also be biotransformed by COX-2, LOXs, or cytochrome P450 into various oxygenated products. Abbreviations: ABHD4, α/β hydrolase domain containing protein 4; ABHDs, α/β hydrolase domain containing proteins 2, 6 or 12; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; CB_1/CB_2: G-protein coupled type-1 and type-2 cannabinoid receptors; COX-2, cyclooxygenase-2; DAGLα/β: diacylglycerol lipase α/β; EMT: putative eCB transmembrane transporter; FAAH: fatty acid amide hydrolase; FABPs, fatty acid binding proteins; GDEs, glycerophosphodiesterases; GPR55/119, G protein-coupled receptor 55 or 119; HSP70, heat shock protein 70; LOXs, lipoxygenases; MAGL: monoacylglycerol lipase; NAPE-PLD: *N*-acylphosphatidylethanolamines-specific phospholipase D; NAAA, *N*-acylethanolamine acid amidase; NAT, calcium-dependent *N*-acyltransferase; PPARs: peroxisome proliferator-activated nuclear receptors α, γ, or δ; THC, Δ^9-tetrahydrocannabinol; TRPs: transient receptor potential channels (V1-3, A1, and M8 types).

14 ECS components [receptors GPR55, GPR119, and TRPV4; enzymes NAT, DAGLα, DAGLβ, GDE1, GDE4, GDE7, ABHD2, ABHD4, ABHD6, and ABHD12; and a putative endocannabinoid membrane transporter (EMT)] are still elusive, thus preventing our understanding of their regulation, crosstalk with other ECS components, and ultimately of their impact on eCB signaling. This information gap is particularly troubling for enzymes involved in 2-AG metabolism: out of the six enzymes discovered so far, only the structure of MAGL is known (Table 2). Therefore, the availability of more structural data appears to be crucial.

A noteworthy example of this need is ABHD2, recently identified as a progesterone-dependent hydrolase in sperm, where 2-AG acts as an endogenous inhibitor of the cation channels also known as CatSper [29]. Upon progesterone stimulation, ABHD2 hydrolyzes 2-AG and leads to CatSper opening and sperm hyperactivation, thus making it fertile [29]. The observation that 2-AG level is controlled by stimulation of its degradation appears of major interest, because it requires a reconsideration of the widely accepted dogma of the on demand synthesis of eCBs; that is, that these compounds (2-AG included) are produced only through the control of their biosynthesis, upon stimulus-dependent release from phospholipid precursors [11]. In sperm, 2-AG is instead degraded on demand from a pre-existing pool [29]. The implications for eCB signaling and for exploitation of ABHD2 as a therapeutic target to treat infertility are apparent. Yet, in the absence of structural data we cannot anticipate how ABHD2 can be tuned by progesterone, and how commonalities and differences between ABHD2 and other ABHDs (i.e., 6 and 12) can make progesterone a general

Lipoxygenase (LOX): a type of ubiquitous nonheme iron enzymes responsible for the oxygenation of arachidonic acid into leukotrienes and lipoxins.
Peroxisome proliferator-activated receptors (PPARs): superfamily of nuclear receptor transcription factors, that include three members: α, γ, and δ. These PPARs are targets of AEA, 2-AG, and their congeners.
Pharmacophore: according to the International Union of Pure and Applied Chemistry (IUPAC), an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response.
Transient receptor potential vanilloid-1 (TRPV1): a six-transmembrane domain receptor channel that is activated by physical and chemical stimuli, as well as by AEA and 2-AG.

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Table 2. Major Elements of the ECS with Known 3D Structure

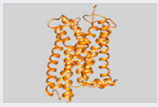
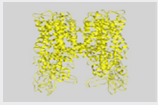
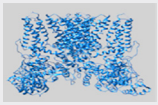
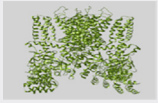
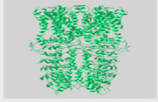
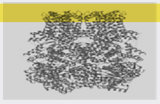
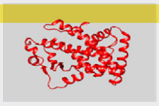
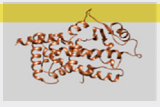
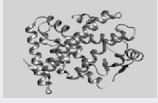
Name	Role in ECS	Localization	PDB code[c]	3D structure	Source material	Refs
CB_1	Receptor/ signaling[a]	Membrane bound	5TGZ		*Homo sapiens*	[49,62]
CB_2	Receptor/ signaling[a]	Membrane bound	5ZTY		*Homo sapiens*	[48]
TRPV1	Receptor/ signaling[b]	Membrane bound	5IRZ		*Homo sapiens*	[63]
TRPV2	Receptor/ signaling[b]	Membrane bound	5HI9		*Rattus norvegicus*	[64]
TRPV3	Receptor/ signaling[b]	Membrane bound	6MHS		*Homo sapiens*	[65]
TRPA1	Receptor/ signaling[b]	Membrane bound	3J9P		*Homo sapiens*	[66]
TRPM8	Receptor/ signaling[b]	Membrane bound	6BPQ		*Homo sapiens*	[67]
PPARα	Nuclear transcription factor	Nucleus	4BCR		*Homo sapiens*	[68]
PPARγ	Nuclear transcription factor	Nucleus	2I4J		*Homo sapiens*	[69]
PPARδ	Nuclear transcription factor	Nucleus	5U3Q		*Homo sapiens*	[70]

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Table 2. ***Continued***

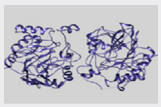
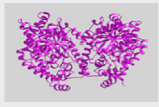
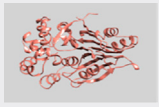
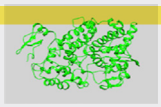
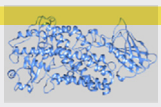
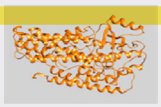
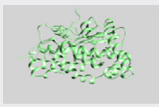
Name	Role in ECS	Localization	PDB code[c]	3D structure	Source material	Refs
NAPE-PLD	AEA biosynthesis	Membrane bound	4QN9		*Homo sapiens*	[71]
FAAH	AEA hydrolysis	Membrane bound	6MRG		*Rattus norvegicus*	[72]
NAAA	AEA and PEA hydrolysis	Lysosomes	6DXX		*Homo sapiens*	[73]
MAGL	2-AG hydrolysis	Cytosol/ membranes	6BQ0		*Homo sapiens*	[56]
COX-2	Conversion of arachidonic acid into prostaglandins	Membrane bound/ mitochondria	4RRW		*Mus musculus*	[74]
5-LOX	Conversion of arachidonic acid into hydroperoxides	Cytosol	3V92		*Homo sapiens*	[75]
12-LOX	Conversion of arachidonic acid into hydroperoxides	Cytosol	3RDE		*Sus scrofa*	[76]
15-LOX	Conversion of arachidonic acid into hydroperoxides	Cytosol	4NRE		*Homo sapiens*	[77]
P450	Conversion of arachidonic acid into epoxy-eicosatrienoic acids	Membrane bound/ mitochondria	2JJN		*Saccharopolyspora erythraea*	[78]
FABP5	Fatty acid binding protein	Cytosol	1B56		*Homo sapiens*	[79]

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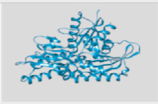
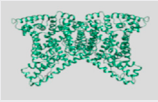
Table 2. *Continued*

Name	Role in ECS	Localization	PDB code[c]	3D structure	Source material	Refs
FABP7	Fatty acid binding protein	Cytosol	1FE3		*Homo sapiens*	[80]
HSP70	Molecular chaperon	Cytosol	1S3X		*Homo sapiens*	[81]
Albumin	Carrier protein	Cytosol	1AO6		*Homo sapiens*	[82]

[a]Extracellular binding site.
[b]Intracellular binding site.
[c]Protein Data Bank (PDB) archive is the single worldwide repository of information about the 3D structures of large biological molecules, such as proteins.

regulator of eCB signaling, nor can we ascertain whether ABHD2 is indeed a distinct target of this hormone.

Regulation of the ECS

Canonical Mechanisms

Receptor–agonist interaction can be modulated by a myriad of mechanisms, that can; (i) increase or decrease the number of receptors, (ii) modify the affinity of the active (orthosteric) site for specific ligands, or (iii) change receptor conformation via positive **allosteric modulators** (PAMs) or negative allosteric modulators (NAMs) that act at nonorthosteric sites. Of note, PAMs and NAMs have been identified for CB_1 [37–39], and among the latter substances are natural noncannabinoid ligands such as pepcans (peptide eCBs) [40] and pregnenolone [41]. Covalent modifications such as lipidation in CB_1 [42], and glycosylation in CB_1, CB_2, and GPR55 [43], have also been shown to modulate receptor activity.

Noncanonical Mechanisms

In addition to the mechanisms reported earlier, there are noncanonical modalities of ECS regulation that take advantage of protein interaction with surrounding lipids in the membrane bilayer. As mentioned earlier, 11 of the known ECS receptors and enzymes are membrane-bound proteins (Table 2). In recent years, it has become apparent that membrane cholesterol plays a distinct role in driving CB_1-dependent signal transduction, by binding to a consensus sequence absent in CB_2 [44]. In particular, location and trafficking of CB_1 [44,45] and its recruitment of selected downstream G proteins [46] depend on a palmitoyl anchor absent in CB_2, marking a clear difference between these two apparently interchangeable targets. As a consequence of these fine-tuned regulations, a **biased signaling** can be triggered, thus leading to different signal transduction and cellular responses [47]. Again, a closer inspection into the structural features of the proteins involved in such a bias will allow us to understand the underlying molecular mechanisms, and to appreciate distinctions among different routes triggered by the same receptor. In this context, recent data on the crystallographic structure of CB_2 appear of major interest, because they show that the extracellular portion of the antagonist-bound CB_2 shares a high degree of conformational similarity with the agonist-bound CB_1 [48]. Though data on agonist-bound CB_2 are still missing, these observations are a proof of concept that an opposing functional profile of CB_2 antagonism versus CB_1 agonism does exist, with clear functional and pharmacological implications [49,50]. The same could hold true

Clinician's Corner

Cannabis (*Cannabis sativa* or *indica*) extracts have been used for centuries in folklore medicine. There is now emerging clinical evidence that some cannabis-derived substances [termed (phyto)cannabinoids], especially THC and cannabidiol, might be effective treatments for neurological disorders, such as severe epilepsy syndromes, pain and spasticity in patients with multiple sclerosis.

The effects of phytocannabinoids seem to largely depend on their interactions with an endogenous network of lipid signals, their receptors, metabolic enzymes, and transporters (collectively known as ECS). Of note, both cannabis extracts and the ECS are complex, and can vary significantly from batch to batch (cannabinoids), and from subject to subject (eCBs).

It is challenging to dissect mutual interactions of phytocannabinoids and eCBs and their impact on human pathophysiology, an aim that cannot be reached without a detailed knowledge of the structural properties and regulation mechanisms of the main elements of the ECS.

Phytocannabinoids are endowed with a huge therapeutic potential, and eCBs may provide therapeutic targets or may be used as disease biomarkers. Yet, detailed knowledge of the structural features of the proteins that bind and/or metabolize these compounds is necessary to understand how they act and eventually use distinct elements of the ECS as novel action points for drug development.

Despite the public perception of cannabis-derived drugs as natural treatments, it is unknown whether phytocannabinoids are indeed safer and better tolerated than other approved therapies, as there are no comparative studies. It is not appropriate to extrapolate the results of trials of standardized preparations to other nonstandardized, nonregulated medical cannabis products.

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for other membrane-bound ECS elements, especially for those with dimeric or tetrameric active forms (Table 2). Indeed, the interaction among subunits can be markedly modulated by membrane lipids, such as cholesterol that promote dimerization and hence inactivation of the β_2-adrenergic receptor [50], as well as dimerization and inactivation of CB_1 [44]. Incidentally, it should be recalled that a high membrane lipid diversity is universal in eukaryotes, and that alterations of membrane lipid homeostasis are linked to various human diseases [51]. Such diversity and alterations that provide distinct regulation of membrane-bound proteins, might contribute to (or even cause) disease conditions. Also, major metabolic enzymes of eCBs, such as NAPE-PLD, FAAH, DAGLα/β, and MAGL, are membrane-bound proteins (Table 2), and thus their availability in the membrane and/or their metabolic activity could be affected by surrounding lipids. In the case of FAAH, it has been recently shown that indeed membrane cholesterol stabilizes a dimeric form of the enzyme, modulates its subcellular localization and enhances its catalytic activity, overall affecting the extent and intracellular localization of the termination of eCB signaling [52]. More recently, *in silico* and *in vitro* experiments have demonstrated additional allosteric sites on the FAAH surface, where major steroid hormones (i.e., testosterone, hydrocortisone, estradiol, pregnenolone, progesterone, and cortisone) can bind and increase enzyme affinity for AEA-containing membranes [53]. This example highlights the importance of knowing the 3D structure in order to investigate the possibilities of modulating ECS metabolic enzymes. Once the 3D structures for 2-AG metabolic enzymes become available, similar data need to be collected. Taken together, it seems apparent that receptors and metabolic enzymes of eCBs can be markedly affected by membrane lipids; an observation that deserves further investigation due to the important implications it may have in fine-tuning eCB signaling. It can be anticipated that the same eCB-binding receptor (e.g., CB_1) or metabolic enzymes (e.g., FAAH) in two different cells, or in the same cells under different lipid-modifying conditions, may behave in different ways, eventually leading to distinct eCB signal transduction. The impact on cell functioning in human health and disease remains to be ascertained. In the same context, unexpected effects of THC and other phytocannabinoids on heterodimerization of $CB_{1/2}$ are emerging [54–56], with a potentially major impact on human diseases (Box 1).

Trafficking of eCBs

On a final note, it is important to recall that eCBs are lipids, and as such they need a suitable carrier to travel the aqueous cytosol, as well as the aqueous extracellular space [13]. Direct evidence for cytosolic AEA-binding proteins has been provided, by showing that the intracellular distribution of tritiated AEA after its uptake is due to a specific, protein-associated AEA-binding activity attributed to heat shock protein (HSP)70 and albumin [57]. Independent overexpression studies also identified fatty acid binding proteins (FABPs) 5 and 7 as additional eCB carriers [58], and more recently FABP1 was added to the list as an intracellular THC transporter [59]. Further data have demonstrated that extracellular AEA [60] and 2-AG [61] transport in the synaptic cleft occurs via microvesicles rather than via protein transporters. Such microvesicle-mediated transport of AEA and 2-AG is instrumental in inhibiting presynaptic transmission in target GABAergic and dopaminergic neurons, respectively [60,61]. While the pathophysiological relevance of intracellular and extracellular trafficking of eCBs remains elusive, it appears that carriers of these lipid signals should be actively sought and investigated, because they might be major players in driving an eCB to the right target, at the right time,

Box 1. Unexpected Effect of Cannabinoids on Heterodimerization of $CB_{1/2}$ Receptors

Heterodimers of the human epidermal growth factor receptor 2 (HER2) with CB_2 have recently been described, and shown to be dissociated by THC binding to CB_2 [54]. It is noteworthy that heterodimers of CB_1 and CB_2 are modulated by cannabigerol, another phytocannabinoid present in cannabis extracts [55]. These findings uncover unexpected modalities of action for THC and other cannabinoids (and possibly also for endocannabinoids) in regulating dimer formation between $CB_{1/2}$ and non-$CB_{1/2}$ receptors, thus opening an entirely new perspective for the design of antitumor therapies for HER2-dependent cancer [54], and other human diseases [55,56]. Thus, the relevance of these findings for proposed clinical indications of phytocannabinoids (Table 1) is evident.

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and in the right concentration, thus holding potential as primary action points for the development of effective and selective eCB-oriented therapeutics.

Concluding Remarks

In this article I have summarized existing evidence that cannabis extracts hold true potential to treat a number of human diseases, both within [2] and outside [10] the nervous system. I have also shown that this potential depends on their ability to modulate an endogenous signaling network made of lipid signals (eCBs), their receptor targets, metabolic enzymes, and intracellular/transmembrane/extracellular transporters, altogether forming the ECS. Overall, available data open up new ground and future perspectives. Yet, it should be appreciated that any cannabis extract can be qualitatively and quantitatively different from another (meaning that its composition can differ in terms of single components and their individual amounts), and the consequences of such differences of the extracts on their pharmacological activity can be dramatic. Therefore, it is of upmost importance to know the structures of the elements (receptors, transporters, and metabolic enzymes) of the ECS before embarking on the study of biological activity and regulation of exogenous and endogenous cannabinoids. An ideal roadmap for future research and therapeutic exploitation of these natural compounds should begin with a clear understanding of the molecular details of the proteins that are engaged in their biological activity, in order to appreciate how fine tuning of them can impact signaling pathways triggered thereof.

In conclusion, the complexity of plant-derived cannabinoids and endogenous ECS appears a 'treasure chest' that only appropriate keys can open and make available for exploitation in human therapy. Molecular details of ECS elements seem to represent the key to unlocking this treasure chest, particularly in the case of emerging noncanonical aspects of their regulation (see Outstanding Questions).

Outstanding Questions

How can we tell which commonalities and differences are responsible for certain biological activities of the ECS without knowing the 3D structure of its major components?

How can we predict the interaction of receptors and metabolic enzymes of eCBs with phytocannabinoids (and their functional consequences) without knowing their 3D structures?

What is the impact of the surrounding lipids on membrane-bound elements of the ECS, and hence on eCB signaling?

Can membrane lipid composition be a relevant driver of the effect of phytocannabinoids and eCBs on receptor homo- and heterodimerization? Can such a membrane lipid-driven receptor dimerization contribute to regulate the biological activity of phytocannabinoids and eCBs?

How do intracellular and extracellular transporters of eCBs contribute to the regulation of their signaling? Can eCB carriers become a major point of action for the development of new therapeutics, based on the fact that they may drive the right eCB to the right target, at the right concentration, and at the right time?

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