New approaches and challenges to targeting the endocannabinoid system

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Abstract | The endocannabinoid signalling system was discovered because receptors in this system are the targets of compounds present in psychotropic preparations of *Cannabis sativa*. The search for new therapeutics that target endocannabinoid signalling is both challenging and potentially rewarding, as endocannabinoids are implicated in numerous physiological and pathological processes. Hundreds of mediators chemically related to the endocannabinoids, often with similar metabolic pathways but different targets, have complicated the development of inhibitors of endocannabinoid metabolic enzymes, but have also stimulated the rational design of multi-target drugs. Meanwhile, drugs based on botanical cannabinoids have come to the clinical forefront, synthetic agonists designed to bind cannabinoid receptor 1 with very high affinity have become a societal threat, and the gut microbiome has been found to signal in part through the endocannabinoid network. The current development of drugs that alter endocannabinoid signalling, as well as how this complex system could be pharmacologically manipulated in the future, are described in this article.

[H1] Introduction

Along with its controversial recreational use, the recent history of psychotropic preparations of *Cannabis sativa* has been marked by their ever increasing, although mostly anecdotal, therapeutic applications for a wide variety of ailments[1]. When, in the mid 1950s and 1960s (refs[2–4]), the first chemical constituents of cannabis were identified, it was imagined that the medicinal activity of this plant could be teased out from its psychotropic effects. The first cannabinoids to be chemically characterised, cannabidiol (CBD)[3] and Δ^9-tetrahydrocannabinol (THC)[4], were the most abundant members of this class of natural products in the dried and heated flowers of *C. sativa* that are used for the production of haemp and marijuana, respectively. Accordingly, CBD was found to be non-psychotropic, whereas THC is responsible for the psychoactive effects of marijuana[5,6].

Despite the fact that CBD and THC were identified at almost the same time, most of the pharmacological efforts were dedicated to understanding the mechanism of action of THC, and, initially, relatively few studies investigated the effects of CBD. This was possibly for two reasons: the need to fully appreciate the potential toxicological and addictive effects of the principal psychoactive component of what had become (and still is) the most widely used illicit drug in western societies, and the fact that the varieties of cannabis used recreationally, in which THC was most abundant, were perceived as the ones with the most promising therapeutic effects. However, there was really no reason to believe that such effects were exclusively caused by THC, nor that preparations from non-psychotropic varieties of cannabis, in which CBD was often most abundant, would not have therapeutic value. Although we now understand that THC acts via two G-protein-coupled receptors (GPCRs) -– the cannabinoid receptor 1 (CB1) and CB2 (refs[7,8]),) - and that CB1 is responsible for the psychoactive effects of marijuana[5,6,9]. , To to date no specific receptor for CBD has been identified, . although However, several different molecular targets have been suggested to mediate distinct pharmacological effects of this cannabinoid. The identification of CB1 and CB2 led to the isolation and characterization of endogenous ligands for these proteins, *N*-arachidonoyl-ethanolamine (AEA) and 2-arachidonoylglycerol (2-AG)[10–12], which were named the endocannabinoids[13], and of five main enzymes for their biosynthesis and inactivation: *N*-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD), diacylglycerol lipases α and β, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)[14–17] (Fig. 1). This system of two signalling lipids, their two receptors and metabolic enzymes became known as the endocannabinoid system and was soon assigned a wealth of physiological roles that went far beyond what could be predicted from the pharmacological actions of THC[18]. Later, alterations in endocannabinoid signalling, due to changes in the expression and function of cannabinoid receptors and endocannabinoid metabolic enzymes, as well as modified endocannabinoid tissue concentrations, were found to be increasingly associated with diverse pathological conditions. Therapies that exploit or correct such alterations might therefore be developed from agonists or antagonists of CB1 or CB2, or from inhibitors of endocannabinoid degradation or biosynthesis[19].

The road to the clinical development of synthetic endocannabinoid system-based drugs (Table 1) has been paved with great hopes and subsequent bitter disappointments. In the meantime, plant cannabinoids, particularly CBD, either alone or in combination with THC, have come back to the limelight as efficacious and relatively safe therapeutic drugs[20,21]. Several possible endocannabinoid-based drugs and their therapeutic applications have been thoroughly reviewed in this journal[19]. Here, we provide some explanation of what has gone wrong so far with these molecules, and why. We also discuss various possible ways out of this impasse, and highlight the role of the gut microbiota as a potential source of information for new endocannabinoid-based therapies.

[H1] Targeting cannabinoid receptors

[H2] CB1 and CB2. CB1 is possibly the most abundant and widespread GPCR in the mammalian brain. This has made the therapeutic use of THC very difficult in pathological conditions for which anecdotal data had previously suggested that marijuana is effective. Subsequent preclinical studies confirmed that the activation of CB1 in peripheral and CNS cells (including but not exclusively neurons) might be beneficial in neuropathic and inflammatory pain, neuropsychiatric disorders including anxiety, depression and PTSD, neurological diseases such as multiple sclerosis and Huntington disease chorea, and inflammatory bowel disorders[22–25]. By contrast, the use of CB1 antagonists for conditions in which CB1 overactivation may contribute to the progression or symptoms of a disease — such as obesity, type 2 diabetes, hepatic or kidney disorders, and even some neurological conditions such as Alzheimer disease and schizophrenia[24,26,27] — has been proved problematicproposed. IndeedHowever, the endocannabinoid system is often activated “on demand” and in a cell-specific and time-specific manner during pathological states to

exert a homeostatic function[19]. Hence, the use of systemic direct agonists and antagonists, which indiscriminately activate or inhibit the function of all CB1 molecules, can interfere with normal CB1 function in non-target cells. Typical examples of unwanted effects of such compounds are the intoxication associated with CB1 agonists (which might also interfere with cognitive functions)[28], and the anxiety and depression caused by CB1 antagonists or inverse agonists[29]. Thus, rimonabant (Acomplia), a CB1 inverse agonist marketed in Europe from 2006 for the treatment of obesity and the metabolic syndrome, had to be withdrawn in 2008 because it induced depression and suicidal ideation in a subset of obese patients[29]. For CB1 activators, marinol (botanical THC) and nabilone (a synthetic THC analogue) are employed for the treatment of cachexia in cancer and AIDS and for chemotherapy-induced nausea, but their very narrow therapeutic window prevents their widespread use[30] (Table 1).

Peripherally restricted agonists and antagonists for CB1 (that is, compounds with very low penetration through the blood-brain barrier) were next designed and found to be devoid of CNS side effects[31]. Efforts have also been dedicated to develop "indirect" agonists: drugs that only act on the populations of receptors that are subject to aberrant defective activation by endocannabinoids. These "indirect" agonists inhibit the inactivation of endocannabinoids by catabolic enzymes (see section below on endocannabinoid metabolism)[32–36] or enhance their activity through positive allosteric modulation[37–39]. These strategies would restore the site-specificity and time-specificity of endocannabinoid activity[19]. Inhibitors of endocannabinoid biosynthesis (see section below on endocannabinoid metabolism)[40–42] and CB1 negative allosteric modulators[43] are in the early stages of development; inhibitors of endocannabinoid biosynthesis have been investigated in preclinical studies of obesity and inflammatory and neuropathic pain [AU:OK?] [44–46]. Recently, the first "biologic" drug in the endocannabinoid field, namacizumab, a negative allosteric modulating antibody that stabilizes the CB1 receptor in an inactive conformation, was submitted for approval to initiate a phase 1a/1b clinical trial for non-alcoholic steatohepatitis (http://www.birdrockbio.com/our-pipeline/namacizumab/), a frequent co-morbidity of abdominal obesity. Importantly, namacizumab, like other biologics, is likely to be peripherally restricted.

CB2 is predominantly expressed in immune tissues and cells, but is also present at low levels in neuronal and non-neuronal (for example, activated microglia) cells of the brain[47–49]. Since its discovery in 1993 (ref.[8]) , it has been seen as a promising target for the treatment of inflammatory and autoimmune diseases, and more recently for liver and kidney fibrosis[47]fibrosis[48–49]. Numerous CB2 agonists have been designed, and several clinical trials have been initiated (Table 1), but few outcomes have been reported[48]. Nevertheless, particularly because it is often overexpressed during pathological conditions in selected cells, CB2 is still considered a potential target for specific, and hence safe, therapeutic drugs[48]. The involvement of this receptor in neurogenesis as well as in neurodegenerative, neuroinflammatory, and, albeit more controversially, neuropsychiatric disorders[49]disorders[47], indicates that future clinical development of CB2 agonists could include these indications.

[H2] "CB3" and other endocannabinoid receptors. The discovery of the first endocannabinoid, AEA[10], in 1992 was soon accompanied by the realization that this compound was rather promiscuous in its molecular targets. AEA and other endocannabinoids bind to L-type Ca^{2+} channels[50,51] to produce non-CB1, non-CB2 mediated effects in vivo[52], and induce GTP-mediated signalling in brain membranes from CB1 knockout mice[53]. In the following decades, pharmacological evidence emerged for the existence of a "CB3" receptor that could mediate some of the effects of AEA, THC and synthetic THC-mimetic aminoalkyilindoles, such as WIN55,212 (refs[53,54]) in the brain, and the effects of a CBD isomer, abnormal-cannabidiol, in vascular endothelial cells[55]. Although these receptors have not yet been characterised, the activity of some orphan GPCRs, such as GPR55 (refs[56,57]) GPR18 (ref.[58]) and GPR110 (ref.[59]) can be modulated by THC, CBD and/or by AEA and some of its congener *N*-acylethanolamines (NAEs), although this evidence is not without some controversy[60,61]. Most of these interactions, even when they occur in vitro at concentrations similar to those necessary for AEA to activate CB1 or CB2, have not been demonstrated to occur in vivo and/or validated through the use of knockout mice. Furthermore, the lack of high affinity radiolabelled ligands for most of these orphan GPCRs has thus far prevented the experimental demonstration of their direct interaction with AEA or cannabinoids, and this interaction has, so far, not been suggested by other structural biology approaches. Other reports have suggested that AEA, and more recently 2-AG, are similar to some non-THC cannabinoids in their capability to bind to GPCRs and ligand-activated ion channels for neurotransmitters, including, for example, serotonin, 5-HT3, glycine and $GABA_A$ receptors (see ref.[62] for a recent review), although the physiopathological relevance of these interactions is yet to be clarified.

From these studies, evidence for the existence of ionotropic receptors that interact with both endocannabinoids and plant cannabinoids has become stronger and stronger. Numerous reports have shown that AEA (and more recently 2-AG) can bind to and activate transient receptor potential vanilloid type-1 (TRPV1) channels both in vitro and in vivo (see ref.[63] for a recent review) **[Au: Which ref should be cited here?]**. These channels were previously considered orphan and believed to be activated only by heat (>42 °C), protons and the hot chilli pepper component, capsaicin. They were later found to also be activated by CBD and other non-THC cannabinoids, such as cannabigerol (CBG) and Δ^9-tetrahydrocannabivarin (THCV) (see the section below on phytocannabinoids), as well as by other long chain fatty acid amides (denoted as *N*-acyl-amides) and esters[64–66]. AEA as well as several plant cannabinoids, including THC, also antagonize melastatin-type 8 TRP (TRPM8) channels, which are activated by menthol and low temperatures. Finally, whereas high micromolar concentrations of AEA are required to activate ankyrin-type1 (TRPA1, the "mustard oil receptor") and vanilloid-type 2 (TRPV2, also activated by heat) TRP channels in vitro, CBD and other plant cannabinoids can produce these effects at submicromolar concentrations (discussed in more detail in the phytocannabinoids section)[67–69].

In summary, endocannabinoids interact with multiple receptors. Therefore, altering the levels of endocannabinoids with the

purpose of indirectly manipulating CB1 and CB2 activity may not be safer or more selective than directly targeting these receptors. In particular, the capability of endocannabinoids to activate TRPV1 channels, which, contrary to CB1 and CB2, exacerbate pain and neurogenic inflammation[63], may explain in part why inhibitors of fatty acid amide hydrolase (FAAH), the enzyme mostly responsible for AEA hydrolysis, have not been successful in clinical trials (discussed below). In fact, AEA has been suggested to be more efficacious at activating human than rat recombinant TRPV1 channels[70,71], and, in preclinical settings, FAAH inhibition often unmasks the TRPV1-mediated effects (for example, proalgesic) of AEA and its NAE congeners[72–75].

[H1] Targeting endocannabinoid metabolism

AEA and 2-AG belong to two large, distinct families of lipids, the NAEs and the 2-acylglycerols (2-AcGs), respectively. AEA is one of the least abundant NAEs, with levels of *N*-stearoyl-, *N*-palmitoyl- and *N*-oleoylethanolamine (SEA, PEA and OEA, respectively) usually at least 10-fold higher in most of the tissues and cells analysed thus far[76]. **[Au: Which ref should be cited here?]** Conversely, 2-AG is among the most abundant 2-AcG.

[H2] Endocannabinoid biosynthesis. The 2-AcGs are synthesized through the sequential action of phospholipase C on phospholipids, and diacylglycerol lipase α (DAGLα) or DAGLβ on the resulting diacylglycerols (DAGs); hence 2-AcGs are derived ultimately from the lipid present at the *sn*-2 position of phospholipids, which is most commonly arachidonic acid (Fig. 2). DAGLα and DAGLβ inhibitors[40–44] are in the early stages of development, and have been suggested to be useful in preclinical studies of obesity and inflammatory pain, respectively[44]respectively[45,4546].

By contrast, NAEs are derived from the processing of *N*-acyl-phosphatidylethanolamines (NAPEs), which in turn are formed by the transfer of an acyl chain from the *sn*-1 position of phospholipids to the -NH_2 group of phosphatidylethanolamines. Arachidonic acid is one of the least abundant esterified fatty acids at the *sn*-1 position, explaining the relatively low levels of AEA compared to other NAEs. NAPEs can be produced by both Ca^{2+}-dependent and Ca^{2+}-independent acyltransferases, and several pathways can convert them to NAEs (Fig. 2). While Ca^{2+}-independent NAPE-synthesizing acyltransferases seem to be predominantly involved in the formation of bioactive saturated and monounsaturated NAEs, such as OEA, PEA and *N*-oleoyl-, *N*-linoleoyl- and *N*-palmitoyl-ethanolamine[77], Ca^{2+}-dependent acyltransferases, as well as distinct NAPE-converting enzymes, have not been selectively assigned to the biosynthesis of any specific NAE[78], and no biosynthetic pathway specific to the generation of polyunsaturated NAEs, such as AEA and *N*-docosahexanoyl-ethanolamine (also known as synaptamide)[59], has been identified so far. As a consequence, AEA is thought to be biosynthesized using the same pathway as other, usually more abundant, NAEs. Likewise, 2-AG may be released together with its congener 2-AcGs, although these are usually less abundant.

NAEs and 2-AcGs other than AEA and 2-AG have receptors and biological effects of their own (Fig. 1), and thus they should not be considered endocannabinoids (see below). Therefore, it may be difficult to manipulate, either pharmacologically or genetically, the biosynthetic pathways of AEA and 2-AG without also interfering with the biosynthesis of related NAEs and 2-AcGs.

[H2] Endocannabinoid catabolism. Similar considerations apply to most endocannabinoid inactivation pathways (Fig. 3). All long chain NAEs are substrates for FAAH, which hydrolyses AEA to arachidonic acid and ethanolamine[32]ethanolamine[16], although this enzyme has less affinity for saturated NAEs than for AEA. *N*-Acylethanolamine acid amidase (NAAA), which has a tissue and cell distribution quite different from that of FAAH, predominantly hydrolyses saturated NAEs, particularly PEA[79]. Furthermore, FAAH also hydrolyses other long chain fatty acid amides, such as the *N*-acyl-taurines and the primary amides (such as the sleep-inducing factor oleamide), which preferably modulate non-CB1, non-CB2 receptors[16,80,81]. Monoacylglycerol lipase (MAGL) is not selective for 2-AG over other 2-AcGs or even other monoacylglycerols[82]; other esterases, such α,β-hydrolase-6 and -12, which have been suggested to catalyse the hydrolysis of endocannabinoids, are likewise nonselective[83]. Oxidation of endocannabinoids occurs via the arachidonate cascade, which includes cyclooxygenase-2 (COX-2)[84], 12- and 15-lipoxygenases[85], and some cytochrome p450 oxygenases[86]. Hence, although NAEs and 2-AcGs with saturated acyl chains are not less likely to be metabolized through oxidation, whereas those with mono- and polyunsaturated chains can be recognized by some of these enzymes.

Several different chemotypes of inhibitors of fatty acid amide hydrolase (FAAH), have been developed[32,33]. At least four of them, PF04457845, BIA 10–2474, V158866 (clinicaltrials.gov ID NCT01748695) and JNJ-42165279 (clinicaltrials.gov ID NCT02432703 and NCT02498392), have been administered to humans, and a fifth one, URB597, is about to be tested in patients with schizophrenia (clinicaltrials.gov ID NCT00916201). PF04457845 was tested in a clinical trial for inflammatory (osteoarthritic) pain, and although it was safe and elevated plasma NAE levels, it was inefficacious[34]. The clinical development of BIA 10–2474 for the treatment of anxiety, Parkinson disease, chronic pain, multiple sclerosis, cancer and hypertension, had to be interrupted following a disastrous trial in which one volunteer was killed and another seriously harmed, for reasons yet to be fully established[35], but likely due to an interaction of the drug with critical targets other than FAAH in the brain[36]. Other FAAH inhibitors tested in humans, such as PF04457845 and V158866, were well tolerated even following extensive testing.

More recently, the development of MAGL inhibitors has also proceeded steadily, and at least two such compounds, ABX-1431 (Abide Therapeutics) and PF-06818883 (Pfizer), have also entered phase I clinical trials. Although promising results were announced for ABX-1431 (http://abidetx.com/news/abide-therapeutics-reports-positive-topline-data-from-phase-1b-study-of-abx-1431-in-tourette-syndrome/), the trial with PF-06818883 was interrupted because of safety reasons (NCT03020784). A strong impetus seems to have been put on producing and testing several chemotypes of inhibitors of MAGL[87] **[Au: Which ref should be cited here?]**and

determining the preclinical efficacy of those that are selective for this enzyme versus those that also inhibit FAAH[88]. This push towards MAGL inhibitors may be the result of the present stand-by of the clinical development of FAAH inhibitors.

The pharmacological and genetic manipulation of endocannabinoid catabolic enzymes is likely to affect other NAEs and 2-AcGs, and hence interfere with signalling pathways outside CB1 and CB2. Indeed, treatment of mice or humans with FAAH inhibitors increases the levels of AEA as well as OEA and PEA[35,89], and often unmasks effects mediated by peroxisome proliferator-activated receptor (PPAR)-α or TRPV1, for which OEA and PEA act as agonists[72–75,90,91,92]. Likewise, inhibition of MAGL elevates the tissue levels of numerous monoacylglycerols, and this may be accompanied by a beneficial metabolic phenotype that is more reminiscent of indirect activation of some of the targets of non-endocannabinoid 2-AcGs (such as the orphan receptor GPR119)[93] than of CB1. Clearly, as shown in most of the studies on selective FAAH and MAGL inhibitors reviewed here, these compounds generally elicit effects that can be antagonised in rodents by CB1 or CB2 antagonists[94,95]. However, a consequence of the redundancy and promiscuity of endocannabinoid catabolic enzymes is that the effects of their inhibition can be unpredictable, although the premise that endocannabinoids are produced and released "on demand" might mitigate some of this unpredictability.

[H2] Metabolic effects of other lipids in these pathwaysOther mediators involved in endocannabinoid metabolism. In addition to being biosynthesized and inactivated together with bioactive congeners, AEA and 2-AG can be produced from, and/or catabolised to, other lipid mediators. A particular species of lysophosphatidic acid (LPA), *sn*-1-lyso-*sn*-2-arachidonoyl-phosphatidic acid, which preferentially activates the GPCRs lysophosphatidic acid receptor 2 (LPA2) as well as LPA1 and LPA3 (ref.[96]), is both a biosynthetic precursor and a metabolic product of 2-AG[97] (Fig. 3). Hydrolysis of 2-AG can also generate arachidonic acid, which is a precursor of prostanoids, although this occurs in an organ-dependent manner (in the brain but not in the gastrointestinal tract)[98,99]. Endocannabinoids are also oxidized by COX-2, and the ensuing endoperoxides are converted by prostaglandin synthases to prostaglandin ethanolamides (prostamides) and glycerol esters (PG-Gs), which may act on their own receptors and often produce pronociceptive and pro-inflammatory effects[100–104]. Thus, manipulation of endocannabinoid metabolic enzymes could not only affect the levels of endocannabinoid congeners, but also those of other biochemically related, but functionally unrelated, lipid mediators. In theory (often supported by experimental evidence), inhibition of DAGLs reduces 2-AG/CB1-CB2 and 2-AG-derived prostanoid or LPA signalling[105], but enhances DAG/protein kinase C signalling. Inhibition of NAPE-PLD, the enzyme catalysing the direct conversion of NAPEs into NAEs[14], reduces AEA signalling to at cannabinoid receptors and TRPV1 channels, as well as NAE signalling to at PPAR-α, but also may also reduce the levels of the other bioactive product of the hydrolytic reaction, phosphatidic acid. Levels of NAPE-PLD substrates are also increased, which have been shown to affect metabolism[106]. Finally, inhibition (or genetic inactivation) of FAAH and MAGL increases the levels of AEA and 2-AG, respectively, but may also increase their oxidation by COX-2 and hence enhance prostamide and PG-G signalling[102,107]. For MAGL, this metabolic shift may also reduce prostanoid[98,99] and enhance LPA[105] receptor activity.

In summary, the evidence outlined above suggests that, as for numerous target pathways, great care must be taken when interpreting studies that pharmacologically manipulate endocannabinoid anabolic and catabolic enzymes. In most cases, generalised knockout of the major endocannabinoid hydrolysing enzymes is accompanied by phenotypes that are sensitive to cannabinoid receptor antagonism (for example, see ref.[108]), and therefore due to elevation of endocannabinoid tissue levels, although MAGL genetic inactivation, due to its subsequent dramatic and congenital elevation of 2-AG levels, a full CB1 agonist, can also produce CB1 desensitization[109,110]. However, cell-specific and time-specific roles for each of these enzymes may exist, possibly during different physiological and pathological conditions. Thus, studies using inducible conditional knockout of the corresponding genes or their mRNA transcripts might cast light on which enzyme needs to be inhibited, and when and where, to selectively affect the levels of endocannabinoids over other related mediators, and hence specifically modulate the activity of one or more endocannabinoid receptors. Such studies will need to include not only the observation of the ensuing phenotypes, but also the quantitative analysis of the tissue (and, when possible, cellular) levels of both endocannabinoids and their related mediators, preferably at different ages and physiopathological settings. Only through the assessment of what lipid mediators are affected, and to what extent, can one surmise what type of receptors can beare modulated by the genetic (or pharmacological) manipulation of endocannabinoid anabolic and catabolic enzymes.

[H1] The "endocannabinoidome"

In the past 15 years, numerous bioactive *N*-acyl-amides have been identified, and their biosynthesis, inactivation and function have been investigated. These putative lipid mediators (Fig. 1) often share receptors and/or catabolic enzymes with endocannabinoids and their congeners, and include: the *N*-acyl-aminoacids (or lipoaminoacids), such as the *N*-acyl-glycines, which are inactivated by FAAH and can activate the orphan GPCR, GPR18, or, as AEA can, inhibit T-type Ca^{2+} channels (Ca_V3s)[58,111–114]; the *N*-acyl-serines, which have important biological effects in vivo and for which anabolic and catabolic pathways, as well as direct targets (other than Ca_V3s)[114], are not yet identified[114–116]; the *N*-acyl-dopamines, most of which activate TRPV1 channels and inhibit FAAH and Ca_V3s[114,117–121]; and the *N*-acyl-serotonins[122], which inhibit FAAH and often antagonize TRPV1 channels, but also inhibit Ca_V3s, and whose biosynthesis so far has been investigated only in *Drosophila*[123–126]. Furthermore, other long chain *N*-acyl-amides, such as *N*-arachidonoyl-tyrosine and *N*-arachidonoyl-tyramine, *N*-acyl-GABAs, -alanines, -methionines, -prolines, -valines, -leucines/isoleucines, -phenylalanines and –carnitines, have been identified[66,127,128], although their metabolism and/or biological effects have not yet been fully investigated. Any bioactive or biogenic amine might be able to form an amide with any long chain fatty acid, thereby generating hundreds of novel bioactive lipids, which could constitute specific signalling signatures responsible for

physiological and pathological phenotypes. Because these novel mediators have numerous chemical features in common with the endocannabinoids, such as inactivating enzymes and molecular targets (Figs. 1,2,3), the name of "endocannabinoidome" was proposed[129–131]. The endocannabinoidome includes these lipid mediators, as well as the aforementioned endocannabinoid congeners (the NAEs and 2-AcGs), the *N*-acyl-taurines, and the fatty acid primary amides; bioactive endocannabinoid metabolites (such as prostamides, PG-Gs, lipoxygenase or cytochrome p450 oxygenase products and *sn*-2-C20:4-LPA) and their biosynthetic precursors (NAPEs, DAGs); and the many molecular targets and metabolic enzymes (several of which are yet unidentified) of all these molecules. Thus, the endocannabinoidome is a considerable expansion from the initial two mediators, five enzymes and two receptors of the endocannabinoid system[129–131].

Predicting the effects of the manipulation of endocannabinoidome enzymes, such as MAGL, FAAH, the DAGLs and NAPE-PLD, is particularly difficult because several endocannabinoidome receptors do not necessarily play similar roles in pathological conditions. For example, TRPV1 and prostamide $F_{2\alpha}$ receptors have opposite effects to CB1 and CB2 on pain, and insulin sensitivity is increased by GPR119, a target for some 2-AcGs and NAEs, and inhibited by CB1, as mentioned above[72,73,90,100–103,132]. Furthermore, GPR55, a proposed (and still controversial) target for endocannabinoids, seems to exacerbate cancer growth, whereas CB1 and CB2 have the opposite effect[130]effect[133,134]. LPA1-3 receptors, again contrary to the effects of CB1 and CB2, contribute to pain and cancer growth[135,136]. GPR55 also seems to activate pain pathways but, contrary to CB1, ameliorates glucose tolerance[137,138]. PPAR-α, for which some NAEs are agonists, and CB1 have opposing roles in regulating lipid accumulation in the liver or in nicotine addiction (inhibitory and stimulatory, respectively)[91,139,140]; and prostamide $F_{2\alpha}$ receptors and CB1 have anti- and pro-adipogenic effects, respectively, in preadipocytes[141].

To further complicate matters, several endogenous and environmental stimuli (such as the relative amounts of different fatty acids in the diet) are predicted to affect the tissue concentrations of several endocannabinoidome mediators, which may act either in concert or in competition with endocannabinoids via non-CB1, non-CB2 targets (including Ca^{2+} channels, TRP channels, PPARs and orphan GPCRs). Therefore, ever more sophisticated, targeted "omics" (transcriptomics, proteomics and lipidomics) methodologies[142,143] are required to study the endocannabinoidome, and to make sense of its overall physiological and pathological role. In particular, two questions will need to be addressed. First, given the commonalities among the regulatory and signalling mechanisms of distinct endocannabinoidome mediators, do these act in concert, with different or redundant roles, to turn on and off cellular responses to external homeostasis-perturbing stimuli, thus resulting in fine physiological tuning or the emergence of pathological states? Secondly, do specific profiles for these hundreds of mediators exist for different individuals or distinct diseases, and will deciphering these signatures lead to new personalised diagnoses and treatments?

[H1] Multi-target drugs

Based on the considerations described in the previous sections, manipulating endocannabinoid levels without affecting other mediators that are related biochemically, although not necessarily functionally, to the endocannabinoids is often difficult. Directly interfering with the activity of CB1, and even CB2, with agonists and antagonists has also been so far problematic. One way to deal with endocannabinoid target promiscuity and metabolic enzyme redundancy is to develop multi-target drugs. For example, since one of the possible consequences of inhibiting endocannabinoid catabolic enzymes is to activate receptors other than CB1 and CB2, which could obstruct any beneficial effects or cause unwanted side effects, new compounds containing one pharmacophore that inhibits FAAH or MAGL and another that antagonises these non-cannabinoid receptors could be therapeutically useful.

Several multi-target drugs that modulate endocannabinoidome receptors and enzymes, either obtained by rational design or found in a serendipitous manner, already exist (Table 2). For example, olvanil, a relatively weak TRPV1 agonist, was originally developed (but never marketed) as an analgesic as it could rapidly desensitize TRPV1 channels, and was later found to also inhibit endocannabinoid transport across the cell membrane[144]. This as yet uncharacterised mechanism for the cellular reuptake of endocannabinoids is necessary for their degradation by intracellular catabolic enzymes[145,146]. Conversely, AM404, initially designed as an endocannabinoid membrane transport inhibitor, was later found to also activate and desensitize TRPV1 (ref.[147]) and, more importantly, to be a metabolic product of acetaminophen (paracetamol) in both rodents and humans[148,149]. The analgesic activity of acetaminophen in rodents was indeed shown to be mediated by indirect activation of CB1 receptors and TRPV1 channel activation or desensitization[150–152]. *N*-arachidonoyl-serotonin, initially designed as a FAAH inhibitor[121] inhibitor[123] and later shown to be an endogenous lipid[122], was found to also antagonize TRPV1 (ref.[123124]) and to have CB1- and TRPV1-mediated analgesic, anxiolytic and antidepressant activity in rodents[123,153–156]. This compound can potentially also be used for inflammatory or irritable bowel disorders[157]. In the attempt to mimic the pharmacophores and improve the stability of *N*-arachidonoyl-serotonin, synthetic piperazinyl carbamates were designed as dual inhibitors of FAAH and TRPV1, and one of them, OMDM-198,[158] was shown to effectively inhibit osteoarthritic pain in rats[159]. These compounds can counteract one of the aforementioned consequences of FAAH inhibition: the indirect activation of TRPV1 channels, which not only exacerbates pain but has also been implicated in anxiety and depression[160,161], two other proposed indications for selective FAAH inhibitors.

As mentioned above, inhibition of AEA hydrolysis can trigger AEA catabolism by COX-2 and the subsequent production of prostamides that, in some cases, have been shown to induce inflammatory and pronociceptive responses, thus counteracting one of the most theoretically promising therapeutic effects of FAAH inhibitors[162]. In this case, dual FAAH-prostamide $F_{2\alpha}$ receptor inhibitors, or dual FAAH-COX inhibitors might be more efficacious at inhibiting inflammatory pain than selective FAAH inhibitors, as shown for some synthetic prototypes of such compounds[163–166]. Interestingly, some previously developed COX inhibitors, the

R-profens, were shown to inhibit the COX-2-mediated peroxidation of endocannabinoids selectively over the peroxidation of arachidonic acid[167], and some derivatives of *R*-flurbiprofen, such as (*R*)-2-(2-Fluorobiphenyl-4-yl)-N-(3-Methylpyridin-2-yl)Propanamide, were found to also inhibit FAAH[168,169]. Functionalization of β-caryophyllene, a naturally occurring CB2 agonist, generated an FAAH- and endocannabinoid substrate-specific COX-2 inhibitor[170]. Adding a COX-blocking moiety to FAAH inhibitors might enhance the analgesic efficacy of FAAH blockade in humans and, notably, inserting a FAAH-blocking feature in COX inhibitors was shown to counteract their damaging effects on the gastric mucosa[171]. Finally, a new generation of pan-prostanoid receptor antagonists with FAAH inhibitory activity produced analgesic activity via mixed antagonism of EP, FP and DP receptors and indirect activation of CB1 receptors[172].

Stronger efficacy can be obtained not only by inhibiting targets that are activated as a consequence of inhibition of FAAH, but also by altering two or more endocannabinoidome proteins involved in a given pathological condition. For example, dual CB1 antagonists/PPARα agonists with nM affinity could potentially be used in metabolic disorders[173]. Furthermore, dual FAAH/MAGL inhibitors, such as JZL195, have been designed and exert stronger effects in several animal models of disease than inhibitors selective for either enzyme alone, and, at least for some indications (Table 2),). they They may be used at lower doses than single MAGL inhibitors, thereby potentially minimizing their tolerance and addictive potential[174–179178].

In summary, while the search for synthetic “magic bullets” has recently waned, there are already several examples of molecules that affect more than one endocannabinoidome target (Table 2). This is not surprising if one remembers that endocannabinoids and other pro-homeostatic endocannabinoidome mediators modulate the activity of multiple targets (Fig. 1). However, whether the endocannabinoidome-based multi-target drugs mentioned in this section exhibit improved efficacy and safety over the corresponding selective compounds still needs to be confirmed in further preclinical studies, and demonstrated in humans.

[H1] Phytocannabinoids

While several pharmaceutical companies were struggling to make CB1 antagonists, CB2 agonists and FAAH inhibitors, active pharmacological research on the neglected cousins of THC, such as CBD, but also THCV, CBG, cannabidivarin (CBDV), cannabidiolic acid (CBDA) and cannabichromene (CBC), started again at the turn of the century. Nabiximols (marketed in the USA as Sativex), the first botanical drug based on plant cannabinoids, consists of a 1:1 ratio of standardised extracts from two different cultivars of *C. sativa* enriched respectively in CBD and THC, and delivers approximately equal doses of THC and CBD together with other minor cannabis components as an oromucosal spray. It has been marketed since 2007 as an efficacious and safe treatment for patients with multiple sclerosis and neuropathic pain (only in Canada) or spasticity (in 30 countries so far)[2621,176179]. Preclinical studies with various combinations of THC and CBD have had positive outcomes[180–182], and nabiximols has also been tested for the treatment of cancer pain, with mixed but promising results[183,184]; the progression of glioblastoma multiforme after recurrence of the tumour, with a seemingly positive outcome (www.gwpharm.com/about-us/news/gw-pharmaceuticals-achieves-positive-results-phase-2-proof-concept-study-glioma); and the main symptoms of Huntington`s disease, with disappointing results in terms of efficacy but not safety[185]. Of note, for pain studies, the numeric rating scale used as a primary endpoint is strongly affected by an individual’s expected effects and greatly varies among individuals, which complicates clinical trials. Previous epidemiological and preclinical studies suggest that CBD, although inactive in animal models of the disease (such as for spasticity in multiple sclerosis)[186], can substantially reduce many of the psychotropic side effects of THC, thus widening its therapeutic window and allowing administration at higher and possibly more effective doses[187–190]. This reduction in the intoxicating effects of THC was not due, as initially hypothesized, to direct antagonism of CB1 receptors (see ref.[191] for review), but rather to a combination of effects on other targets, such as adenosine A_{2a} and 5-HT_{1a} receptors[189,–191].

Another non-psychotropic phytocannabinoid, THCV, reduced disease in preclinical models of obesity and insulin resistance[192,193], which was partly confirmed in a phase II clinical trial[194]. These effects were suggested to occur via interaction with several targets, rather than, as initially hypothesized, through neutral antagonism of CB1. The antipsychotic effects of CBD in patients with schizophrenia may occur through non-CB1 targets[195,196], and the anti-convulsant effect of CBD in phase III clinical trials in paediatric patients with Dravet or Lennox-Gastaut syndrome[20,197] is also probably not CB1-mediated, as pro-convulsant activity has occasionally been observed with synthetic CB1 agonists and antagonists[198].

Indeed, unlike THC, the pharmacology of non-psychotropic phytocannabinoids seems to be driven by interactions with more than one receptor or enzyme[199]. This combination of molecular mechanisms of action might explain why CBD is quite safe in humans (doses up to 800 mg/day were administered in one of the two schizophrenia clinical trials[195]) and efficacious in most of the preclinical studies in which this compound has been tested. Recently, a model has been developed that attempts to explain how the multi-target nature of non-psychotropic phytocannabinoids fits successfully and safely with the multi-factorial and multi-receptor nature of several diseases, including pain, spasticity, epilepsy and inflammatory bowel disorders. This model enables the prediction of whether a “therapeutic handshake” occurs between the etiopathological handprint of these disorders and the polypharmacological handprint of some phytocannabinoids, and can be applied in principle to other non-selective natural and synthetic drugs[200]. If supported by mathematical and other bioinformatics approaches, and together with systems biology-guided methodologies, the “therapeutic handshake” model might predict that existing multi-target molecules are safe and effective in unexpected conditions - thus leading to their repositioning- and help to rationally design new multi-target drugs[200].

The clinical success of nabiximols, and possibly CBD (Epidiolex, was currently being considered forrecently approval approved by the FDA), which was effective in following successful clinical trials for the treatment of orphan paediatric epilepsies[20,197],), might

further boost the development of other phytocannabinoids, as more than 100 such metabolites are found in the flowers of different varieties of *C. sativa*. These metabolites interact with receptors and enzymes of the endocannabinoidome (Table 3 and ref.[201] for a recent review). For example, as already mentioned, phytocannabinoids, particularly CBD, CBG and THCV, activate and desensitize TRPV1 channels much in the same way as NAEs, *N*-acyltaurines, *N*-acyldopamines and other *N*-acyl-amides do. CBD was reported to antagonize GPR55, which was reported to be activated by endocannabinoids and PEA (although, as discussed above, this is still controversial). THC was recently shown to activate GPR18, an effect also suggested for *N*-arachidonoyl-glycine. Finally, both phytocannabinoids and *N*-acyl-amides may interact with PPARs (see ref.[202] for a recent review). Although phytocannabinoids also interact with non-endocannabinoidome proteins, the partial overlap between the phytocannabinoids and the endocannabinoidome might facilitate the rational design of new synthetic endocannabinoidome-based drugs, starting from the chemical structures of multi-target phytocannabinoids and endocannabinoidome mediators. This could be especially useful when phytocannabinoids and endocannabinoidome mediators if endocannabinoidome-targeted drugs cannot be clinically developed because of stability, pharmacokinetics or marketing issues.

[H1] Synthetic cannabinoids

Whereas plant cannabinoids, either as purified compounds (CBD) or as standardised botanical extracts (nabiximols), are achieving success in the clinic, synthetic cannabinoids with ultra-potent agonist activity at CB1 receptors have started obtaining a different type of fame. THC is a relatively high affinity but inefficacious agonist for both CB1 and CB2, even compared to endocannabinoids like 2-AG, and can therefore be considered a partial agonist[203]. Indeed, the partial agonist activity of THC, combined with the presence of CBD and other non-psychotropic, multi-target phytocannabinoids, likely explains why this compound, at the relatively low concentrations usually found in traditional, 1960s-style cannabis preparations, does not exhibit strong addictive properties[187]. On the other hand, some synthetic cannabinoids or chemically unrelated cannabimimetic compounds, such as the aminoalkylindoles, exhibit up to 5-fold higher efficacy in most in vitro functional assays for CB1 activity[204,205]. As such, these compounds can produce much stronger effects, especially in adolescents, in view of the important role played by CB1 receptors in neural development, synaptic plasticity and cognition, but also in cardiovascular function (see refs[206,207] for recent reviews). Several chemical variations of these synthetic drugs have further increased their potency. These ultra-potent CB1 agonists, which can be made in large amounts with inexpensive starting materials and relatively simple organic reactions, are becoming a very serious problem because of their often dangerous side effects, ranging from agitated delirium, hallucinations, tachycardia and hypertension to psychosis, seizures, lethargy and coma[208,209], occasionally leading to fatalities, which may also be the result of interactions with targets other than CB1 (ref.[210]). Compounds detected in eight synthetic cannabinoid-associated deaths in adolescents revealed that five of them had no other discernible cause of death on autopsy, and exhibited detectable plasma levels of synthetic CB1 agonists such as PB-22 (1.1 ng/mL), JWH-210 (12 ng/mL), XLR-11 (1.3 ng/mL), AB-CHMINACA (8.2 ng/mL), UR-144 (12.3 ng/mL), and JWH-022 (3 ng/mL)[210]. Such levels may yield concentrations from ~2 to 20 nM, which, for these compounds, would be sufficient to fully activate CB1 receptors.

The scale and rapidity of the evolution of these new recreational drugs (more than 150 chemical structures are known to date) make their legal control and analytical detection in biological samples difficult, if not impossible[208]. Therefore, their consumption is on the rise, and it is estimated that between 3 and 10% of high school students in the USA use these compounds[210]. From a chemistry perspective, nNew efforts are thus urgently required to increase the analytical capabilities of forensic institutions in order to allow the identification of new chemical entities as soon as they appear on the market, to understand potential dangerous side effects of these chemicals and find potential pharmacological treatments for serious cases of intoxication from these compounds. For example, a single administration of dismissed CB1 antagonists such as rimonabant and taranabant, which were safely administered with relative overall safety to thousands of healthy volunteers and obese patients in clinical trials[26,29,211], might block the acute effects of synthetic cannabinoids if their consumption can be deemed responsible for life-threatening effects in hospitalised individuals. These Conversely, ultra-potent synthetic CB1 agonists are unlikely to lead to clinically useful compounds, given the difficulties of harnessing THC for therapeutic use. In fact, their ever increasing abuse may cast a shadow on the future therapeutic use of plant cannabinoids, thus prolonging the partly unjustified stigma that has affected this class of natural compounds since the mid-1960s.

[H1] Endocannabinoids and gut microbiota

The gut microbiota is increasingly known to be involved in a plethora of physiopathological conditions (see ref.[212] for a recent review). Interestingly, a probiotic, *Lactobacillis acidophilus*, was shown to produce antinociceptive effects against visceral pain by increasing the expression of CB2 and MOR1 opioid receptors in intestinal epithelial cells in rats with colonic hypersensitivity[213]. However, from this report it was not clear whether the effects of the probiotic were direct or through alterations in the gut microbiota, as are many of its other effects. Later, several papers showed that increased gut permeability (similar to that induced by obesity), which causes intestinal dysbiosis and thus leads to lipopolysaccharide (LPS)-induced inflammation, can occur through activation of CB1 receptors[214]. Accordingly, in a subsequent study, the protective effects of capsaicin, the prototypical TRPV1 agonist and hot chilli pepper component, against obesity-induced and dysbiosis-mediated low grade inflammation in mice were found to be accompanied by downregulation of colonic CB1 expression[215]. Activation of CB1 with a synthetic agonist eliminated the beneficial effects of capsaicin on body weight, food intake, glucose tolerance and fat accumulation[216]accumulation[215]. These data suggest that CB1 signalling by endocannabinoids acts as one of the intermediate actors between high fat diet-induced dysbiosis and its low grade inflammation-mediated negative effects of on metabolism.

The relationship between the endocannabinoid system and the gut microbiota seems to be bi-directional. Blockade of CB1

receptors was recently suggested to ameliorate diet-induced obesity and accompanying dysmetabolism by increasing the relative abundance of *Akkermansia muciniphila*, a commensal Gram-negative bacterium with beneficial effects on metabolism[213]metabolism[216], and subsequently reducing inflammation[217]. These data indicate that the enhanced peripheral CB1 signalling usually associated with obesity and related disorders (see refs[31,132,218] for recent reviews) may result in aberrant metabolism by facilitating dysbiosis, and suggests the existence of a vicious circle between enhanced CB1 signalling and dysbiosis — this cycle is induced by a high fat diet and amplifies low grade inflammation and the metabolic syndrome. Consistent with this hypothesis, chronic administration of THC, which may lead to CB1 receptor internalization and desensitization[219], counteracts diet-induced obesity and concomitantly alters the gut microbiota, increasing the relative amount of *A. muciniphila*[220]. Interestingly, however, in another report, selective depletion of NAPE-PLD in white adipose tissue induced obesity, glucose intolerance, adipose tissue inflammation and dyslipidemia[221]. This phenotype was caused by inflammation of white adipocytes, which then became less responsive to cold-induced browning, and hence to cold-induced energy expenditure. These alterations were mediated by a shift in gut microbiota composition (and increased gut permeability), and hence could be partially transferred to wild type germ-free mice[221]. These data are counterintuitive, as NAPE-PLD is the biosynthetic enzyme for AEA and its deletion might be expected to reduce CB1 activity and produce beneficial metabolic effects. However, the authors also showed that adipocyte-specific NAPE-PLD knockout mice did not have reduced endocannabinoid tone in the white adipose tissue, but rather reduced levels of NAEs (namely SEA, OEA and PEA, but not AEA)[221]; hence the observed phenotype was possibly due to impaired signalling from receptors (i.e. GPR119, TRPV1 and PPARα,) rather other than from CB1. This is the first study to suggest an important role for some non-endocannabinoid lipid mediators of the endocannabinoidome in protecting from the deleterious effects of dysbiosis on gut permeability and metabolic control. Furthermore, the study exemplifies how experiments with conditional knockout of endocannabinoidome metabolic enzymes must always be accompanied by the understanding of which endocannabinoidome mediator is affected, and where.

An intestine-specific knockout of myeloid differentiation primary response gene 88 (MyD88) also linked intestinal inflammation with the endocannabinoidome, following the quantification of metabolites. MyD88 is a central adaptor molecule for the majority of toll-like receptors through which microbiota-derived inflammatory molecules (such as LPS) signal to the immune system, and has been suggested to mediate the function of the gut microbiota in energy homeostasis. Mice lacking MyD88 in epithelial intestinal cells were protected from diet-induced obesity and insulin resistance and had reduced hepatic steatosis, fat mass and inflammation, increased energy expenditure, improved glucose homeostasis and an increased number of regulatory T cells in the intestine[222]. Protection was transferred to wild-type germ-free mice following gut microbiota transplantation - indicating that the beneficial effects of MyD88 deletion were mediated by alterations in the intestinal microbiome - and was accompanied by decreased levels of AEA and increased levels of 2-AcG (including 2-AG) and increased expression of GPR119 in the intestines of mice fed a high fat diet[222]. Increased 2-AG levels seem contradictory to the role of CB1 in promoting the dysmetabolic and pro-inflammatory effects of dysbiosis. However, decreased CB1 tone, via lower AEA levels, and increased GPR119 and CB2 tone, via higher levels of 2-oleoyl-glyerol or 2-palmitoyl-glycerol and 2-AG, respectively, may explain the beneficial effects on insulin resistance and the reduction in inflammation. Accordingly, another study showed that the beneficial effects of *A. muciniphila* on metabolism, gut permeability and inflammation are accompanied by increased intestinal levels of 2-AG and 2-AcG[223].

The altered gut microbiome has not only been implicated in metabolic disorders. The excessive use of antibiotics and the subsequent perturbation of the intestinal flora have been suggested to play a role in neuropsychiatric disorders such as autism, psychoses, anxiety and depression[224]. A recent study suggested that alterations in some endocannabinoidome mediators might be partly responsible for some of these effects in mice[225]. In two experimental tests of depression, perturbation of the microbiota by prolonged treatment with antibiotics was accompanied by an inflammatory state and strong behavioural changes, altered BDNF/TrkB signalling and neuronal firing and increased microglial inflammatory activity in the hippocampus. Simultaneously, the levels of two *N*-acyl-serotonins that inhibit FAAH and TRPV1 were substantially reduced in the small intestine. This finding, given the suggested role of these two endocannabinoidome proteins in depression (see above), and the capability of *N*-acyl-serotonins to cross the blood-brain barrier[226], suggested that the observed depression-like signs were due in part to disinhibited central FAAH and TRPV1 activity. In support of this hypothesis, administration of a probiotic counteracted the antibiotic-induced behavioural and CNS functional alterations and concomitantly restored near-physiological levels of intestinal *N*-acyl-serotonins[225]. The mechanism through which the antibiotics reduced the levels of these compounds remains to be established, and the contribution of microbial species to the biosynthesis of *N*-acyl-serotonins should be explored. Indeed, another recent study suggested that gut bacterial species can produce small molecules that are chemically very similar to some endocannabinoidome mediators and can interact with the same receptors, thus potentially affecting metabolic disorders[227]. Additionally, endocannabinoidome mediators that are typically produced by the host, such as OEA and PEA, could also be produced by the microbiome and might modulate the effects of dysbiosis. For example, production of these lipids could increase in response to dysbiosis and subsequently decrease gut barrier permeability by stimulating PPARα and TRPV1 channels, thereby counteracting inflammation[228].

In summary, components of the endocannabinoidome likely modulate some of the effects of the gut microbiome, particularly in the context of host-microorganism interactions that alter immune and intestinal function, metabolic control and behaviour[212,224]. Understanding such interactions is likely to suggest new therapeutic drugs for dysbiosis-related diseases, for example through the identification of bioactive metabolites that are produced by commensal microorganisms alone or in collaboration with the host (that is, “post-biotics”). New endocannabinoidome-based medicines may come from these efforts.

[H1] Conclusions and outlook

Drugs targeting the endocannabinoid system, particularly if viewed as part of a larger signalling system — the endocannabinoidome — already exist and new ones are being developed. Several widely used therapeutic interventions have been found to affect the

endocannabinoidome and likely act partly through it (reviewed in ref.[229]). For example, acetaminophen, one of the most popular antipyretic and anti-inflammatory drugs on market, is increasingly suggested to act in part through the endocannabinoidome; the *R*-profens may owe part of their analgesic effects to reduced COX-2-mediated peroxidation of endocannabinoids; and ketamine may also act partly through these mediators[230]. Finally, the increasing success of botanical drugs based on non-psychotropic phytocannabinoids is likely due, at least in part, to their simultaneous modulation of several endocannabinoidome proteins[200]. Indeed, most endocannabinoidome lipids modulate the activity of multiple targets (Fig. 1), and formulations of at least one of them, PEA, have been efficacious in clinical trials of chronic and inflammatory pain, dermatological conditions, intraocular pressure in glaucoma and others[231]. All this should not come as a surprise, since several successful therapeutic drugs that have been on the market for years (such as many NSAIDS, tyrosine kinase inhibitors and metformin) are now known to be promiscuous in their mechanism of action, and polypharmacology is increasingly used to treat in a more efficacious and safer manner several multi-factorial disorders.

Therefore, there is a strong rationale for developing synthetic or natural multi-targeted drugs for from the endocannabinoidome. However, efforts will also need to be dedicated to other strategies, such as the clinical development of some of the >100 *C. sativa* cannabinoids whose pharmacology is now being investigated. Endocannabinoidome receptors that recognize fewer endogenous ligands, such as CB2 (for which even AEA is only a partial agonist)[232], and that are selectively expressed in tissues or cells during pathophysiological conditions, also need to be studied more thoroughly. While waiting for effective and safe non-brain penetrant analogues, CB1 antagonists could be repositioned for use in patients with a low risk of developing depression relative to obese individuals, or for orphan and otherwise untreatable diseases. CB1 antagonists could be useful in treating muscular dystrophies, for example, given CB1 anti-differentiating activity in muscle cells[233]. Existing and new CB2 agonists, or, possibly even better, dual CB1 antagonists/CB2 agonists[234], might be extremely useful to treat fibrosis of the liver, lung, heart and kidney[44]kidney[49,45]. Exploiting the positive and negative allosteric sites in CB1 and CB2 (ref.[23537-39]) could be useful in diseases such as chronic pain, cancer, anxiety, depression, schizophrenia, and metabolic and neuroinflammatory disorders. In this respect, recently identified endogenous negative allosteric modulators of cannabinoid receptors, like some haemopressin analogues and pregnenolone[235–238], could provide starting points for new treatments for addiction disorders and cannabis and synthetic cannabinoid intoxication. Finally, the thorough biomolecular investigation of gut microbiome-endocannabinoidome interactions will likely lead to new drugs, which could include synthetic analogues of multi-target endocannabinoidome mediators designed to have drug-like properties.

We now understand that the endocannabinoid system is complex. However, the plethora of potential therapeutic opportunities that it offers is precisely because of this complexity. Multiple methodologies and technologies, such as the “omics” as well as bioinformatics and systems biology approaches suitable to deal with, and make sense of, “big data”, are now available and will allow us to cope with most of the complications described in the present article, much in the same way they are now proposed to be used for precision and personalised medicine. This should push drug developers to look at this field as a challenging and exciting goldmine, rather than a “no-go” area.

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FIGURE LEGENDS

Fig. 1 | **Endocannabinoidome mediators and receptors.** The main endocannabinoids, AEA and 2-AG, are part of larger families of lipids, the NAEs and the 2-AcGs, respectively. Numerous other members of these families signal through other GPCRs, ion channels and transcription factors as shown. In addition, long chain primary fatty acid amides, lipoaminoacids and acyl neurotransmitters signal through some of the receptors used by NAEs and 2-AcGs, but also some others. **[Copy ed: Please add abbreviations]**

Fig. 2 | **Synthesis of the endocannabinoidome mediators.** Although NAPE-PLD and DAGLα or DAGLβ are considered to be the main enzymes involved in the biosynthesis of the NAEs and 2-AcGs, respectively, numerous other potential biosynthetic pathways exist. In addition, some of the precursors are signalling lipids themselves. Among the *sn*-1-lysophospholipids, only lysophosphatidylinositols have shown to activate GPR55 (ref.[60]) . Limited knowledge is available on the biosynthesis of *N*-acyl-neurotransmitters, and only in *Drosophila*, where the corresponding neurotransmitters play important roles. Similarly, little is known about the biosynthesis of lipoaminoacids, except for *N*-acyl-glycines, whose anabolic processes, and role as precursors of primary amides, are well understood **[Copy ed: Please fix and add abrreviations]** Abbreviations are defined in the main text, except for: AANATL2, arylalkylamine *N*-acyltransferase-like 2; ABHD4, α,β-hydrolase of type 4; D1-3, dopamine receptors 1–3; DHEA, *N*-docosahexaenoyl-ethanolamine; GDE1, glycerophosphodiesterase E1; GLYATL3, glycine *N*-acyltransferase-like 3; GPR110, orphan G-protein coupled receptor 10; 5-HTx, serotonin receptors of different types; LEA, *N*-linoleoyl-ethanolamine; 2-LG, 2-linoleoyl-glycerol; NATs, *N*-acyl transferases; 2-OG, 2-oleoyl-glycerol; PA, phosphatidic acid; PAM, peptidylglycine α-amidating monooxygenase; PLA1, phospholipase A1; PLC, phospholipase C; PLD, phospholipase D; sPLA2, soluble phospholipase A2.

Fig. 3 | **Catabolism of the endocannabinoidome mediators.** Oxidizing enzymes (medium blue) and more pathway-specific serine hydrolases are primarily responsible for the catabolism of endocannabinoidome signalling molecules. FAAH and MAGL are thought to be the primary enzymes responsible for the catabolism of AEA and 2-AG respectively. Products of oxidation (indicated in red) often signal through GPCRs as shown. The thickness of the arrows indicates the importance of the pathway in metabolite degradation. NAEs are shown in light blue, 2-AcGs in grey, lipoaminocids in light green, and the acyl neurotransmitters in pink. FAAH (dark blue) catabolises multiple endocannabinoidome signalling molecules. The receptor for prostamide $F_{2\alpha}$ has been suggested to be an heterodimer between the prostaglandin $F_{2\alpha}$ FP receptor and its splicing variant 4 (FP Alt4)[239]. Several *N*-acylethanolamines, including AEA, as well as 2-AG, have been also suggested to have nonspecific intracellular carriers (not shown in the figure) such as fatty acid-binding proteins[241] and heat shock protein-70 (ref.[242]), which seem to be important for their inactivation. **[Copy ed: Please fix and add abrreviations]** Abbreviations are defined in the main text or in Fig. 1, except for: ABHD6, α,β-hydrolase of type 6; ABHD12, α,β-hydrolase of type 12; COMT, catechol-*O*-methyl-transferase; DP, prostaglandin D_2 receptor; EP, prostaglandin E_2 receptors; FAAH-2, fatty acid amide hydrolase type-2; LOX, lipoxygenase; MAGK, monoacylglycerol kinase; LT, leukotriene receptors; P2Y6, P2Y purinoceptor 6; p450, cytochrome p450 oxygenase; 2-PG, 2-palmitoyl-glycerol; PG, prostaglandin; PGE_2-G, prostaglandin E_2-glycerol.

Table 1 | Selected clinically tested, synthetic and botanical endocannabinoid system-based drugs

Drug	Mechanism of action	Indication(s)	Current status	Reasons for limited use or failure	Refs
Synthetic CB1 or CB2 modulators					
Nabilone (Cesamet)	CB1 CB2 agonism	Nausea and emesis in cancer patients	Marketed in the USA and elsewhere	Narrow therapeutic window	243
Rimonabant (Acomplia)	CB1 inverse agonism/antagonism	Obesity, type 2 diabetes, dyslipidemia	Withdrawn	Psychiatric side effects (depression, anxiety, suicidal ideation) in target patient population	244
GW842166	CB2 agonism	Pain	Terminated	No efficacy in clinical trials	245
S-777469	CB2 agonism	Atopic dermatitis	Phase II recently completed	NA	246 NCT00703573
JBT-101	CB2 agonism	Systemic lupus erythematosus, scleroderma, dermatomyositis, cystic fibrosis	Phase III ongoing (systemic scleroderma); phase II ongoing or completed for other indications	NA	247 NCT03398837, NCT03451045, NCT02466243, NCT03093402, NCT02465437
APD371	CB2 agonism	Abdominal pain in Crohn's disease	Phase IIa recruiting	NA	NCT03155945
Namacizumab	CB1 negative allosteric modulation	Non-alcoholic steatohepatitis	Planned (www.birdrockbio.com/our-pipeline/namacizumab/)	NA	
SAB378	CB1,CB2 agonism (peripherally restricted)	HIV-associated neuropathy	Terminated	NA	248
NEO1940	CB1, CB2 agonism (peripherally restricted)	Cancer and anorexia or weight loss associated with cancer	Phase I study completed, further development announced	NA	http://neomed.ca/en/2018/01/30/artelo-biosciences-neomed-institute-enter-exclusive-global-option-license-agreement/
Synthetic endocannabinoid metabolism modulators					
PF-04457845	FAAH inhibition	Osteoarthritic pain	Phase II study completed	No substantial effect on primary endpoint	34
URB-597	FAAH inhibition	Symptoms of schizophrenia	Phase I. Not yet recruiting	NA	NCT00916201
V158866	FAAH inhibition	Spinal cord injury-induced neuropathic pain	Completed	Failed to meet primary endpoint	NCT01748695
JNJ-42165279	FAAH inhibition	Social anxiety disorders Major Depressive Disorder With Anxious Distress	Recruiting	NA	NCT02432703 NCT02498392
BIA 10-2474	FAAH inhibition with other targets	Anxiety, Parkinson's disease, chronic pain, cancer, hypertension	Phase II study discontinued	One death and mild to severe injury in 4 other subjects in a phase II clinical trial	3435,3536
PF-06818883	MAGL inhibition	Cerebral haemorrhage	Phase I trial discontinued	Safety issues	NCT03020784
ABX-1431	MAGL inhibition	Tourette's syndrome; neuromyelitis optica, neuralgia, myelitis, neuropathies, multiple sclerosis	Phase Ib trial completed. Encouraging results communicated by the developers (http://abidetx.com/news/abide-therapeutics-presents-positive-data-from-a-phase-1b-study-of-abx-1431-in-tourette-syndrome-at-the-american-academy-of-neurology-70th-annual-meeting/)	NA	NCT03138421, NCT03447756
Phytocannabinoids					
THC (dronabinol, Marinol)	CB1 agonism, CB2 partial agonism	Nausea and emesis in patients with cancer, wasting syndrome in patients with AIDS	Marketed in the USA and elsewhere	Narrow therapeutic window	240
Botanical THC+CBD (nabiximols; Sativex in the USA)	CB1 agonism, CB2 partial agonism (see TABLE 3 for other targets of CBD)	Spasticity and pain in multiple sclerosis; glioblastoma; cancer pain	Marketed in over 30 countries for spasticity in multiple sclerosis	NA	4921,179,183,184

Botanical CBD (for example, Epidiolex and other formulations)	Multi-target (see TABLE 3)	Refractorypediatric epilepsies (Dravet's and Lennox-Gastaut's syndrome), schizophrenia	Undergoing consideration by the FDA for orphan pediatric epilepsies	NA	4820,195–197,249
THCV	Multi-target (see TABLE 3)	Type 2 diabetes and dyslipide-mia	Two phase II studies completed	Primary endpoints were not met	194

NA, not applicable

Table 2 | Selected marketed[a] and preclinical drugs with multiple therapeutically relevant targets including components of the endocannabinoidome

Drug	Cannabinoid receptor and endocannabinoid metabolic enzyme targets	Other endocannabinoidome receptor and metabolic enzyme targets	Potential therapeutic use	Refs
Acetaminophen (via AM404 and other metabolites)[a]	• FAAH (-) • eCBmt (--)	• TRPV1 (++) • TRPA1 (++) • COX2 (-; ns, nss)	Pain, fever	147-152,250, 251
Dipyrone (via its AM404-like metabolites)[a]	• CB1 (+) • CB2 (+)	TRPV1 (+) COX2 (-; ns, nss)	Pain, fever	251
ARN2508	FAAH (---)	COX2 (---; ns, nss)	Pain without gastric mucosal damage, IBDs	170171
OMDM198	FAAH (-)	TRPV1 (--)	Inflammatory pain, anxiety	158,159
OMDM202	FAAH (-)	TRPA1 (++)	Inflammatory pain	158
AGN211335, AGN211336	FAAH (--)	Heterodimer between the FP receptor and its Alt4 splicing variant	Inflammatory pain	163
(R)-2-(2-Fluorobiphenyl-4-yl)-N-(3-Methylpyridin-2-yl)Propanamide	FAAH (-)	COX2 (-; ns, ss)	Inflammatory pain, anxiety	169
Compound "11e"	• FAAH (-) • eCBmt (-)	COX2 (-; ss)	Inflammatory pain, anxiety	171170
Compound "11f"	• CB2 (+) • eCBmt (-)	COX2 (-; ss)	Inflammatory pain, anxiety	1710
AGN220653	FAAH (--)	Various prostanoid receptors (DP1, DP2, EP1, EP4, FP, TP)	Inflammatory pain	172
Compound "2" and compound "3"	CB1 (--)	PPARα	Metabolic syndrome, hepatosteatosis	173
JZL195	• FAAH (--) • MAGL (---)	None	Neuropathic and visceral pain, nausea, pruritus and depression (but not anxiety disorders)	174-178,88

Abbreviations not already defined in the text or in Figures: ns, non-selective over COX-1: nns, non-substrate selective for 2-AG over arachidonic acid oxidation by COX-2; ss, substrate selective for 2-AG over arachidonic acid oxidation by COX-2. Legend: -, mild inhibition; - -, moderate inhibition: - - -, strong inhibition; +, mild activation; ++, moderate activation; +++, strong activation. [a]Drugs that have been tested in clinical trials and are currently marketed.

Table 3 | Proposed key molecular targets for the most studied non-psychotropic phytocannabinoids

pCB	CB1 and CB2	TRP channels	PPARs and orphan GPCRs	Endocannabinoidome enzymes and transporters	Neurotransmitter receptors and voltage-dependent ion channels	Potential therapeutic uses	Refs
CBD	NAM for CB1[a]	TRPV1 (+), TRPV2 (+), TRPV3 (+)[a], TRPA1 (+), TRPM8 (-)	PPARγ (+), GPR55 (-), GPR3 (-)[a], GPR6 (-)[a], GPR12 (-)[a]	FAAH (-), ENT (-), eCBmt (-)	5-HT_{1a} (+), Gly (+), $GABA_A$ (+)[a], Ca_V3 (-), Ca_V1 (-), $Na_V1.6$ (-)[a], VDAC-1 (-)[a]	Chronic and inflammatory pain, epilepsy, IBDs, schizophrenia, cancer, neuroinflammatory diseases	64,67,68,199,202,251,252, 257–260261
CBDV	None	TRPV1 (+), TRPA1 (+), TRPM8 (-)	None	DAGLα (-) [a], eCBmt (-)	None	Epilepsy	67,68,199,257
CBDA	None	None	PPARγ (+)	DAGLα (-) [a], NAAA (-)[a]	PAM for 5-HT_{1a} [a]	Nausea, cancer	199,202,254,255, 257
THCV	CB1 (-), CB2 (+)	TRPV1 (+), TRPV2 (+), TRPV3 (+)[a], TRPA1 (+), TRPM8 (-)	None	None	5-HT_{1a} (+)[a]	Obesity, metabolic syndrome, insulin resistance, steatosis, schizophrenia, inflammatory pain	67,199,253,257,258
CBG	None	TRPV1 (+), TRPV2 (+), TRPA1 (+), TRPM8 (-)	PPARγ (+)	eCBmt (-)[a]	$α_2$ (+)[a], 5-HT_{1a} (-)[a]	Cancer, neurodegenerative diseases, IBDs	67,202,254,256-257,258
CBC	None	TRPA1 (+)	None	ENT (-)[a,b], eCBmt (-)[a]	None	Pain, gliosis	67,199,257,261
THCA	None	None	PPARγ (+)	DAGLα (-) [a], MAGL (-)[a]	None	Neurodegenerative diseases	67,69,254,257

Abbreviations not already defined in the text or in Figures: $α_2$, $α_2$ adrenergic receptor; $GABA_A$, γ-aminobutiric acid receptor A; Gly, glycine receptors; ENT, equilibrative nucleoside transporter; NAM, negative allosteric modulator, $Na_V1.6$, voltage-activated sodium channel type 1.6; THCA, $Δ^9$-tetrahydrocannabinolic acid; PAM, positive allosteric modulator; pCB, phytocannabinoid; VDAC-1, Voltage-dependent anion channel type 1. Legend: (+) activation; (-), inhibition. [a]based only on one study; [b]based only on in vivo pharmacological data.

Figure 1

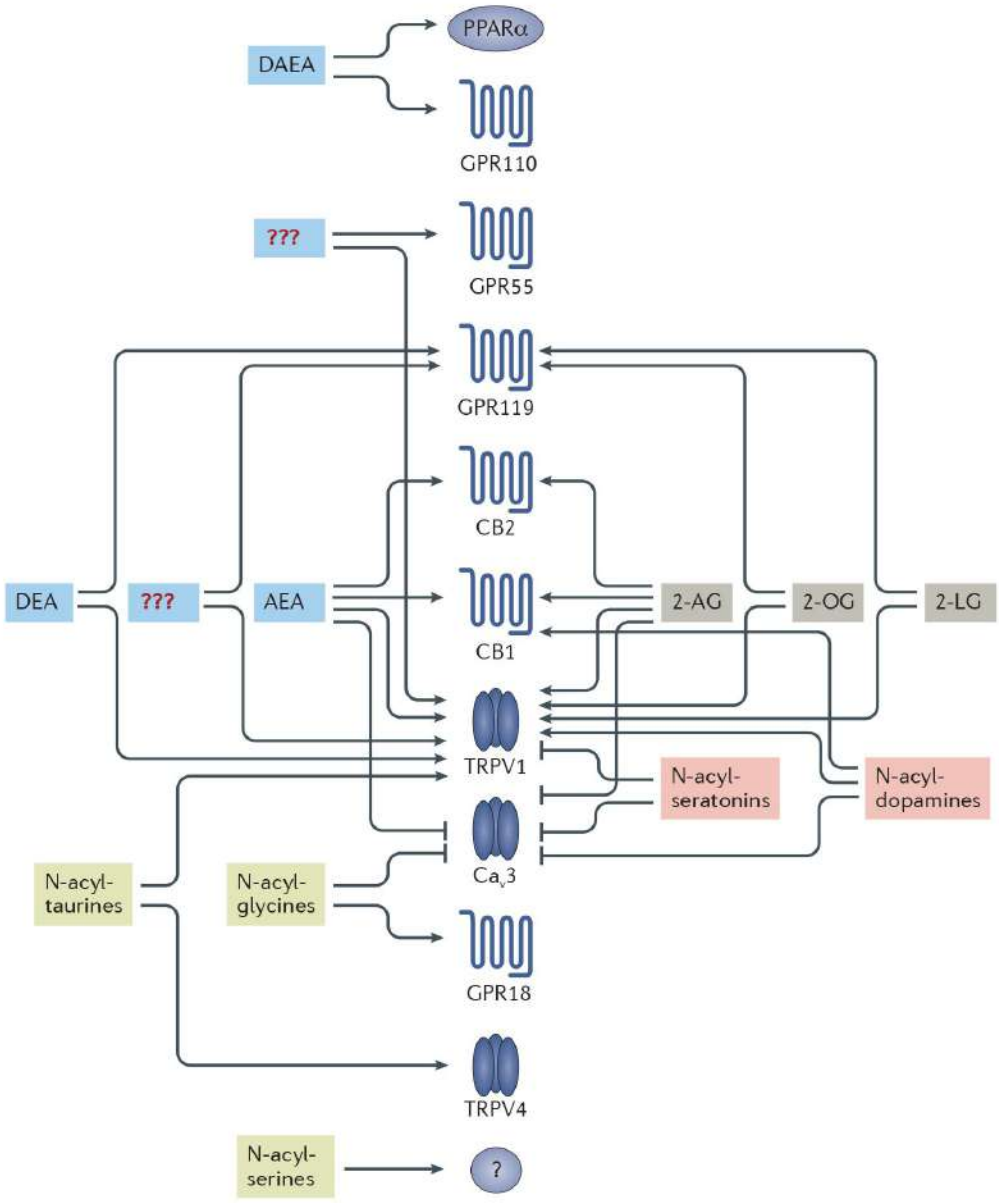

PPARα
DAEA
GPR110
???
GPR55
GPR119
CB2
DEA
???
AEA
CB1
2-AG
2-OG
2-LG
TRPV1
N-acyl-
seratonins
N-acyl-
dopamines
Cav3
N-acyl-
taurines
N-acyl-
glycines
GPR18
TRPV4
N-acyl-
serines
?

Figure 2

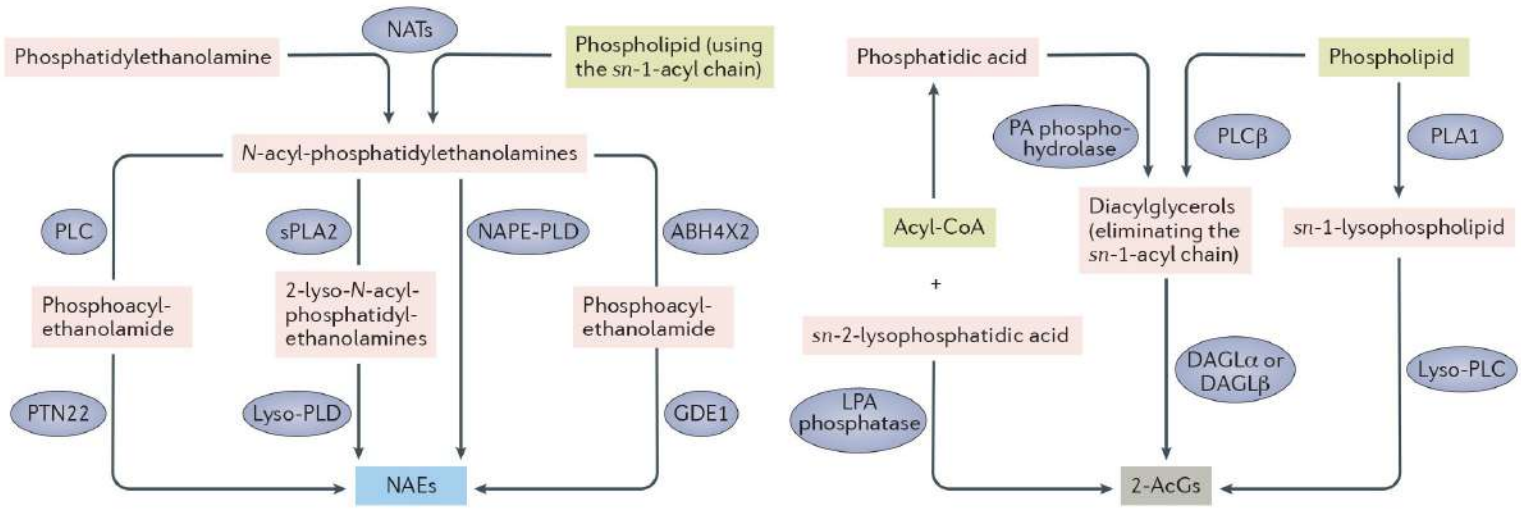
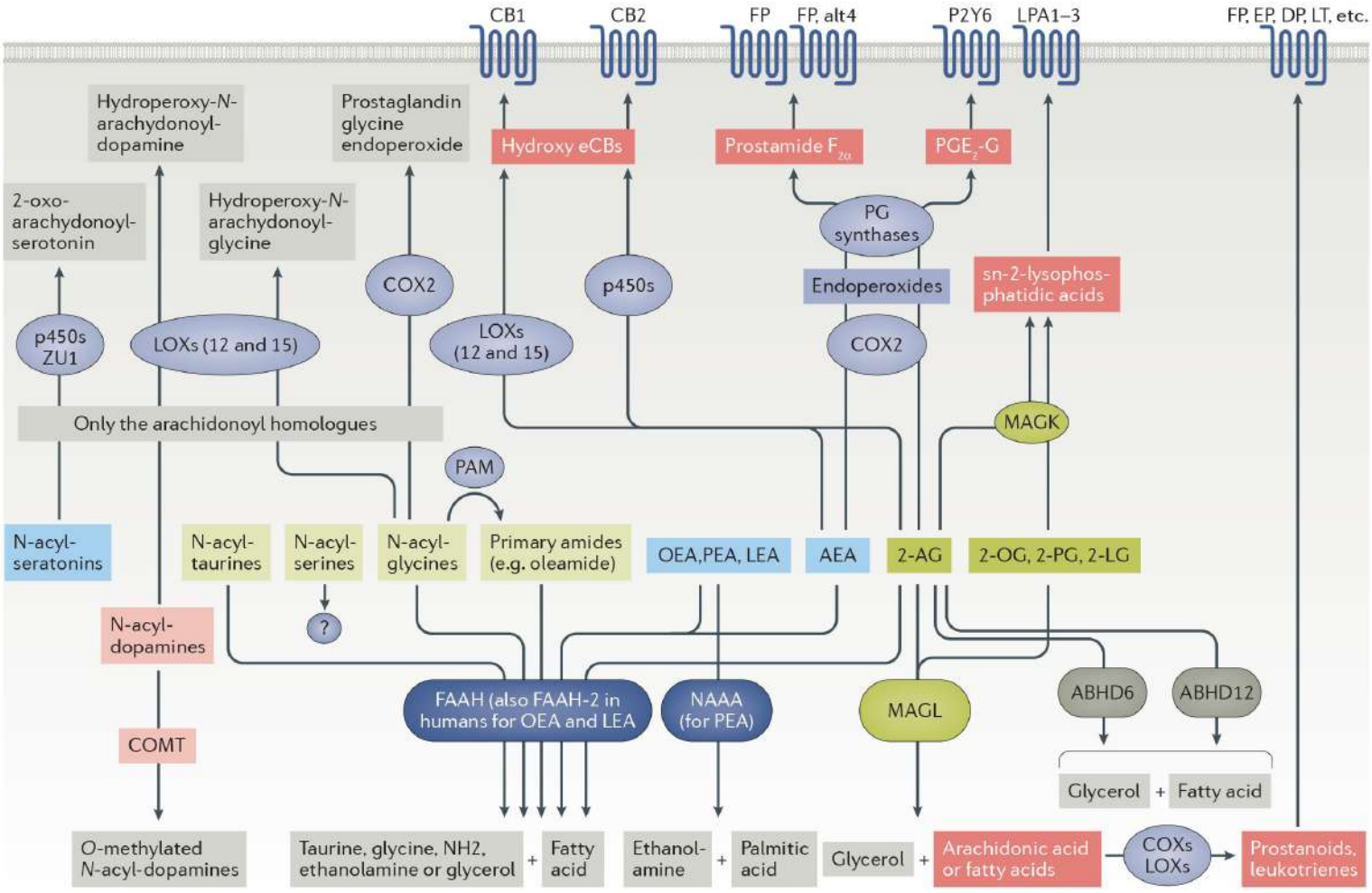

NATs
Phosphatidylethanolamine
Phospholipid (using the sn-1-acyl chain)
N-acyl-phosphatidylethanolamines
PLC
sPLA2
NAPE-PLD
ABH4X2
Phosphoacyl-ethanolamide
2-lyso-N-acyl-phosphatidyl-ethanolamines
Phosphoacyl-ethanolamide
PTN22
Lyso-PLD
GDE1
NAEs
Phosphatidic acid
Phospholipid
PA phospho-hydrolase
PLCβ
PLA1
Acyl-CoA
Diacylglycerols (eliminating the sn-1-acyl chain)
sn-1-lysophospholipid
+
sn-2-lysophosphatidic acid
DAGLα or DAGLβ
Lyso-PLC
LPA phosphatase
2-AcGs

Figure 3


CB1
CB2
FP
FP, alt4
P2Y6
LPA1–3
FP, EP, DP, LT, etc.
Hydroperoxy-N-arachydonoyl-dopamine
Prostaglandin glycine endoperoxide
Hydroxy eCBs
Prostamide F2α
PGE2-G
2-oxo-arachydonoyl-serotonin
Hydroperoxy-N-arachydonoyl-glycine
PG synthases
COX2
p450s
Endoperoxides
sn-2-lysophos-phatidic acids
p450s ZU1
LOXs (12 and 15)
LOXs (12 and 15)
COX2
Only the arachidonoyl homologues
MAGK
PAM
N-acyl-seratonins
N-acyl-taurines
N-acyl-serines
N-acyl-glycines
Primary amides (e.g. oleamide)
OEA,PEA, LEA
AEA
2-AG
2-OG, 2-PG, 2-LG
N-acyl-dopamines
?
FAAH (also FAAH-2 in humans for OEA and LEA
NAAA (for PEA)
MAGL
ABHD6
ABHD12
COMT
Glycerol + Fatty acid
O-methylated N-acyl-dopamines
Taurine, glycine, NH2, ethanolamine or glycerol + Fatty acid
Ethanol-amine + Palmitic acid
Glycerol + Arachidonic acid or fatty acids
COXs LOXs
Prostanoids, leukotrienes