OPINION

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 and how this complex system could be pharmacologically manipulated in the future are described in this Opinion article.

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[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 characterized, 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* varieties that are used for the production of hemp 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. We now understand that THC acts via two G protein-coupled receptors (GPCRs) — cannabinoid receptor 1 (CB1) and CB2 (REFS[7,8]) — and that CB1 is responsible for the psychoactive effects of marijuana[5,6,9]. However, to date, no specific receptor for CBD has been identified. 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] (FIG. 1), which were named the endocannabinoids[13], and of five main enzymes for their biosynthesis and inactivation: *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD), *sn*-1-specific diacylglycerol lipase-α (DGLα), DGLβ, fatty acid amide hydrolase 1 (FAAH) and monoacylglycerol lipase (MAGL; also known as MGL)[14–17]. This system of two signalling lipids, their two receptors and their 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, owing 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 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 bitter disappointments. In the meantime, plant cannabinoids, particularly CBD, either alone or in combination with THC, have come back into the limelight as efficacious and moderately safe therapeutic drugs[20,21]. Several endocannabinoid-based drugs and their therapeutic applications have been thoroughly reviewed in this journal[19]. Here, I provide some explanation of what has gone

wrong so far with these molecules and why. I 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.

Targeting cannabinoid receptors

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 central nervous system (CNS) cells (including but not exclusively neurons) might be beneficial in neuropathic and inflammatory pain; neuropsychiatric disorders including anxiety, depression and post-traumatic stress disorder; neurological diseases such as multiple sclerosis and Huntington disease chorea; and inflammatory bowel disorders[22–25]. By contrast, the use of CB1 antagonists or inverse agonists 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 proposed. However, 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 or inverse agonists, 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 in 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 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 (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 act on only the populations of receptors that are subject to 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].

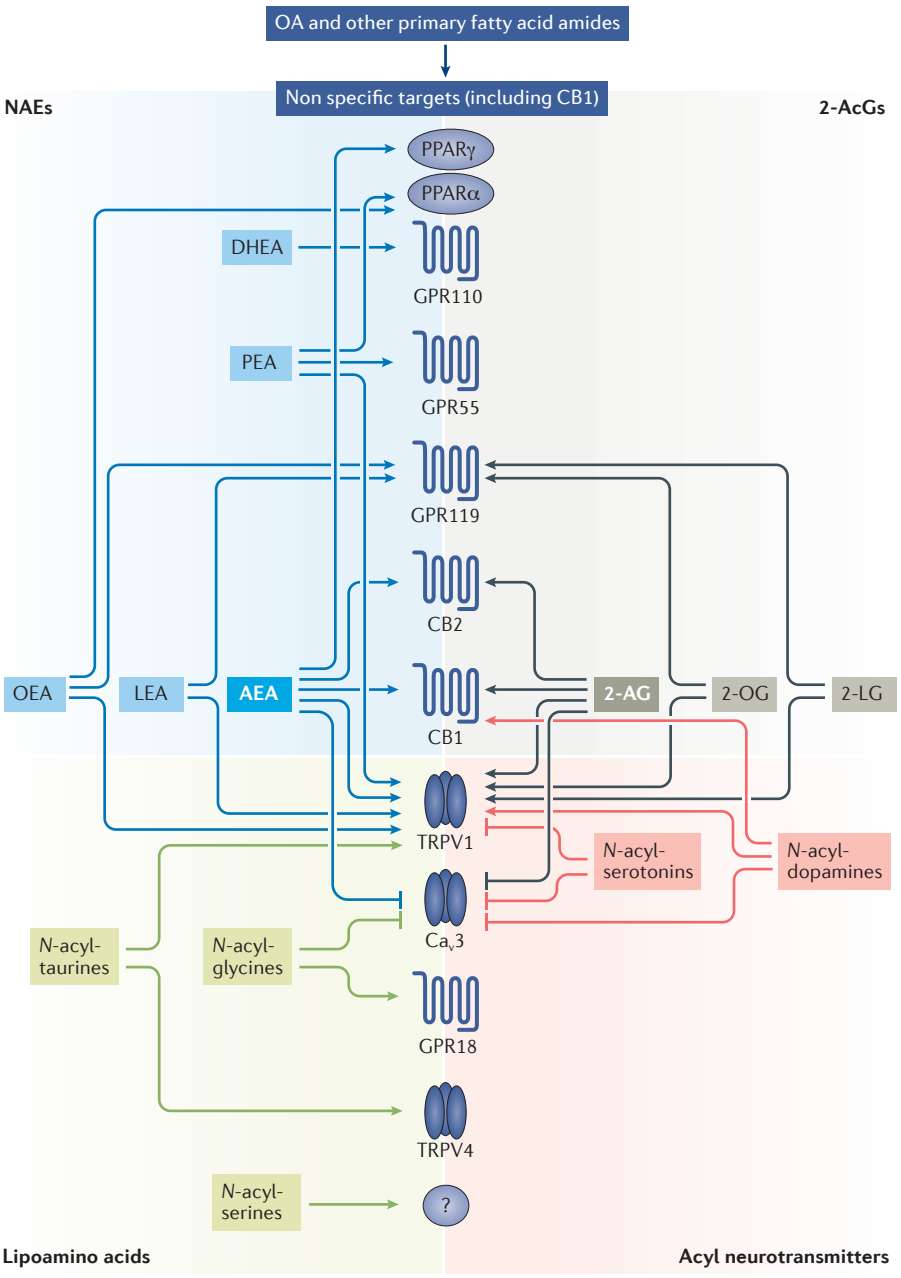

Figure 1 | **Endocannabinoidome mediators and receptors.** The main endocannabinoids, *N*-arachidonoyl-ethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), are part of larger families of lipids, the *N*-acylethanolamines (NAEs) and the 2-acylglycerols (2-AcGs), respectively. Numerous other members of these families signal through other G protein-coupled receptors (GPCRs), ion channels and nuclear receptors, as shown. In addition, long-chain primary fatty acid amides, lipoamino acids and acyl neurotransmitters signal through some of the receptors used by NAEs and 2-AcGs. 2-LG, 2-linoleoyl glycerol; 2-OG, 2-oleoyl glycerol; Ca_v3, T-type Ca^{2+} channel; CB, cannabinoid receptor; DHEA, *N*-docosahexaenoyl ethanolamine; GPR18, G protein-coupled receptor 18; LEA, *N*-linoleoyl ethanolamine; OA, oleamide; OEA, *N*-oleoylethanolamine; PEA, *N*-palmitoylethanolamine; PPAR, peroxisome proliferator-activated receptor; TRPV1, transient receptor potential cation channel subfamily V member 1.

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

Drug	Mechanism of action	Indications	Current status	Reasons for limited use or failure	Refs and ClinicalTrials.gov identifiers
Synthetic CB1 or CB2 modulators					
Nabilone (Cesamet)	CB1 and CB2 agonism	Nausea and emesis in patients with cancer	Marketed in the USA and elsewhere	Narrow therapeutic window	[242]
Rimonabant (Acomplia)	CB1 inverse agonism and/or antagonism	Obesity, type 2 diabetes and dyslipidaemia	Withdrawn	Psychiatric side effects (depression, anxiety and suicidal ideation) in target patient population	[243]
GW842166	CB2 agonism	Pain	Terminated	No efficacy in clinical trials	[244]
S-777469	CB2 agonism	Atopic dermatitis	Phase II trial recently completed	NA	[245] NCT00703573
JBT-101	CB2 agonism	Systemic lupus erythematosus, scleroderma, dermatomyositis and cystic fibrosis	Phase III trial ongoing (systemic scleroderma) and phase II trial ongoing or completed for other indications	NA	[246] NCT03398837, NCT03451045, NCT02466243, NCT03093402 and NCT02465437
APD371	CB2 agonism	Abdominal pain in Crohn's disease	Phase IIa trial recruiting	NA	NCT03155945
Namacizumab	CB1 negative allosteric modulation	Nonalcoholic steatohepatitis	Phase I trial planned	NA	[247]
SAB378	CB1 and CB2 agonism (peripherally restricted)	HIV-associated neuropathy	Terminated	NA	[248]
NEO1940	CB1 and CB2 agonism (peripherally restricted)	Cancer and anorexia or weight loss associated with cancer	Phase I trial completed and further development announced	NA	[249]
Synthetic endocannabinoid metabolism modulators					
PF-04457845	FAAH inhibition	Osteoarthritic pain	Phase II trial completed	No substantial effect on primary end point	[34]
URB597	FAAH inhibition	Symptoms of schizophrenia	Phase I trial not yet recruiting	NA	NCT00916201
V158866	FAAH inhibition	Spinal cord injury-induced neuropathic pain	Completed	Failed to meet primary end point	NCT01748695
JNJ-42165279	FAAH inhibition	Social anxiety disorders, major depressive disorder with anxious distress	Phase II trial recruiting	NA	NCT02432703 and NCT02498392
BIA 10-2474	FAAH inhibition with other targets	Anxiety, Parkinson disease, chronic pain, cancer and hypertension	Phase II trial discontinued	One death and mild to severe injury in four other subjects in a phase II clinical trial	[35,36]
PF-06818883	MAGL inhibition	Cerebral haemorrhage	Phase I trial discontinued	Safety issues	NCT03020784
ABX-1431	MAGL inhibition	Tourette syndrome, neuromyelitis optica, neuralgia, myelitis, neuropathies and multiple sclerosis	Phase Ib trial completed; encouraging results communicated by the developers	NA	[250] NCT03138421 and NCT03447756
Phytocannabinoids					
THC (dronabinol; Marinol)	CB1 agonism and CB2 partial agonism	Nausea and emesis in patients with cancer and wasting syndrome in patients with AIDS	Marketed in the USA and elsewhere	Narrow therapeutic window	[251]

Table 1 (cont.) | **Selected clinically tested, synthetic and botanical endocannabinoid system-based drugs**

Drug	Mechanism of action	Indications	Current status	Reasons for limited use or failure	Refs and ClinicalTrials.gov identifiers
Phytocannabinoids (cont.)					
Botanical THC + CBD (nabiximols; Sativex in the USA)	CB1 agonism and CB2 partial agonism (see TABLE 3 for other targets of CBD)	Spasticity and pain in multiple sclerosis, glioblastoma and cancer pain	Marketed in over 30 countries for spasticity in multiple sclerosis	NA	21,179,183,184 NCT01812603 and NCT01812616
Botanical CBD (for example, Epidiolex and other formulations)	Multi-target (see TABLE 3)	Refractory paediatric epilepsies (Dravet and Lennox–Gastaut syndromes) and schizophrenia	Approved by the FDA for the treatment of convulsions in Dravet and Lennox–Gastaut syndromes	NA	20,195–197,252
THCV	Multi-target (see TABLE 3)	Type 2 diabetes and dyslipidaemia	Two phase II trials completed	Primary end points were not met	194

CB, cannabinoid receptor; CBD, cannabidiol; FAAH, fatty acid amide hydrolase 1; MAGL, monoacylglycerol lipase; NA, not applicable; THC, Δ^9-tetrahydrocannabinol; THCV, Δ^9-tetrahydrocannabivarin.

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[44–46]. Recently, the first biologic drug in the endocannabinoid field, namacizumab, a negative allosteric antibody that stabilizes CB1 in an inactive conformation, was submitted for approval to initiate a phase 1a/b clinical trial for nonalcoholic steatohepatitis (see Related links), a frequent comorbidity 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]. 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[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[47] indicates that future clinical development of CB2 agonists could include these indications.

Other endocannabinoid receptors. The discovery of the first endocannabinoid, AEA[10], in 1992 was soon accompanied by the realization that this compound had numerous molecular targets. AEA and other endocannabinoids bind to L-type Ca^{2+} channels[50,51], produce other non-CB1-mediated and non-CB2-mediated effects in vivo[52] and induce GTP-mediated signalling in brain membranes from *Cnr1*-knockout mice[53]. In the following decades, pharmacological evidence emerged for the existence of other receptors that could mediate some of the effects of AEA, THC and synthetic THC-mimetic aminoalkylindoles — such as WIN 55,212 (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 characterized, the activity of some orphan GPCRs, such as GPCR 55 (GPR55)[56,57], GPR18 (REF.[58]) and GPR110 (also known as ADGRF1)[59], can be modulated by THC, CBD and/or AEA and/or 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 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 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, glycine and the $GABA_A$ receptor ($GABA_AR$) (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 increased. Numerous reports have shown that AEA (and more recently 2-AG) can bind to and activate transient receptor potential cation channel subfamily V member 1 (TRPV1) both in vitro and in vivo (see REF.[63] for a recent review). These channels were previously considered orphan and believed to be activated by only 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 and several plant cannabinoids, including THC, also antagonize TRP cation channel subfamily M member 8 (TRPM8), which is activated by menthol and low temperatures[63,67]. Finally, whereas high micromolar concentrations of AEA are required to activate TRPA1 (the mustard oil receptor) and TRPV2 (also activated by heat) 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, which, contrary to CB1 and CB2, exacerbates pain and neurogenic inflammation[63], may explain in part why inhibitors of 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 (REFS[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].

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*-stearoylethanolamine (SEA), *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA) usually at least tenfold higher in most of the tissues and cells analysed thus far[76]. Conversely, 2-AG is among the most abundant 2-AcGs.

Endocannabinoid biosynthesis. The 2-AcGs are synthesized through the sequential action of phospholipase C (PLC) on phospholipids and DGLα or DGLβ 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). DGLα and DGLβ 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[44–46].

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

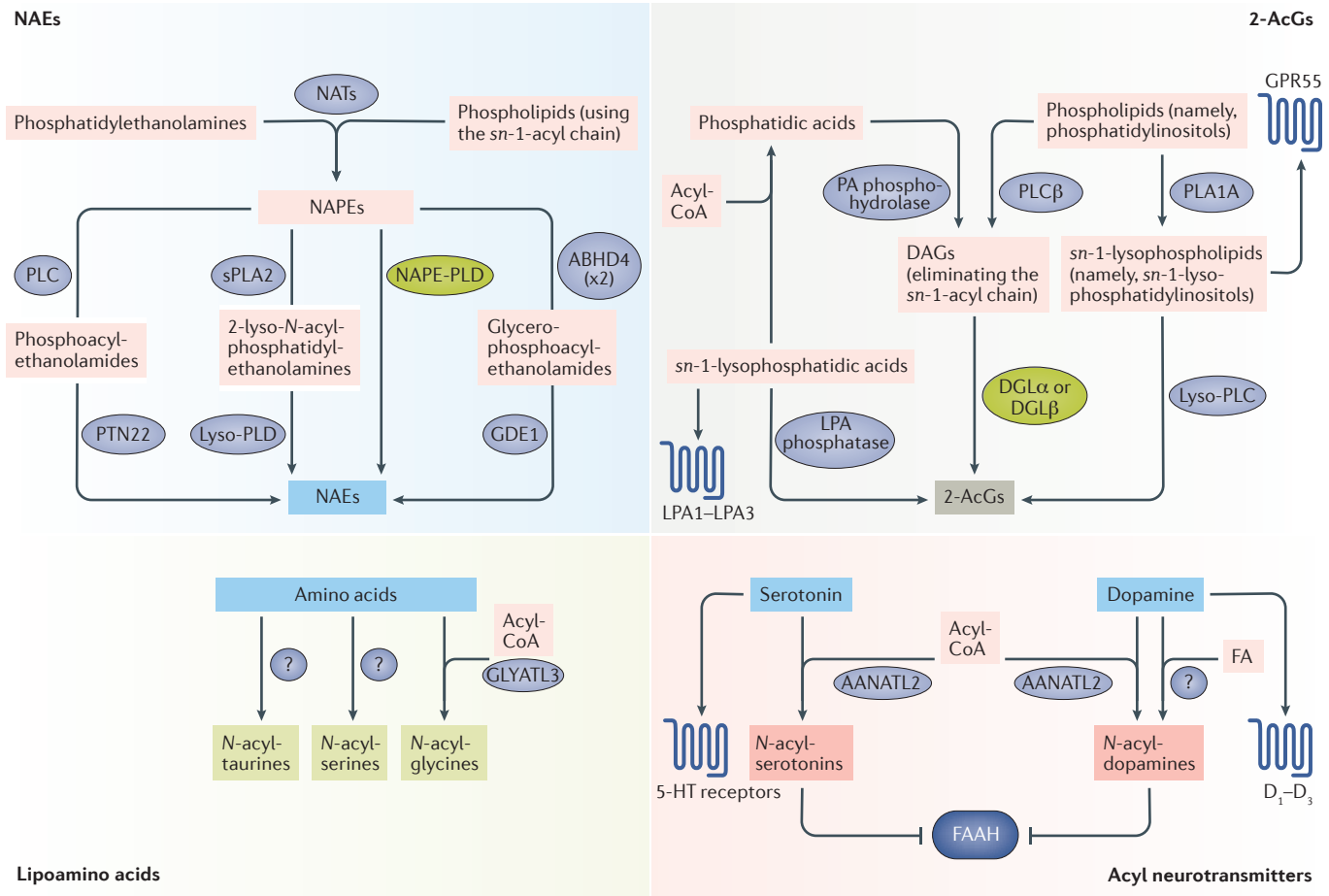

Figure 2 | **Synthesis of the endocannabinoidome mediators.** Although *N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD) and *sn*-1-specific diacylglycerol lipase-α (DGLα) or DGLβ are considered to be the main enzymes involved in the biosynthesis of the *N*-acylethanolamines (NAEs) and 2-acylglycerols (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 been shown to activate G protein-coupled receptor 55 (GPR55)[60]. Limited knowledge is available regarding the biosynthesis of *N*-acyl neurotransmitters, and this has only been studied in *Drosophila melanogaster*, in which the corresponding neurotransmitters play important roles. Similarly, little is known about the biosynthesis of lipoamino acids, except for *N*-acyl-glycines, for which the anabolic processes and their role as biosynthetic precursors of primary amides are well understood. 5-HT, 5-hydroxytryptamine; AANATL2, arylalkylamine *N*-acyltransferase-like 2, isoform A; ABHD4, α/β-hydrolase 4; D_1, dopamine receptor 1; DAGs, diacylglycerols; DHEA, *N*-docosahexaenoyl ethanolamine; FA, fatty acid; FAAH, fatty acid amide hydrolase 1; GDE1, glycerophosphodiester phosphodiesterase 1; GLYATL3, glycine *N*-acyltransferase-like protein 3; LPA, lysophosphatidic acid; LPA1, LPA receptor 1; NAPEs, *N*-acyl-phosphatidylethanolamines; NATs, *N*-acyltransferases (including phospholipase A2 group IVE and phospholipase A/acyltransferase 1); PA, phosphatidic acid; PLA1A, phospholipase A1 member A; PLC, phospholipase C; PTPN22, tyrosine-protein phosphatase non-receptor type 22; sPLA2, soluble phospholipase A2.

abundant esterified fatty acids at the *sn*-1 position, explaining the relatively low levels of AEA compared with 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). Although 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*-linoleoyl-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).

In summary, 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.

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[16], although this enzyme has less affinity for saturated NAEs than for AEA. *N*-Acylethanolamine-hydrolysing acid

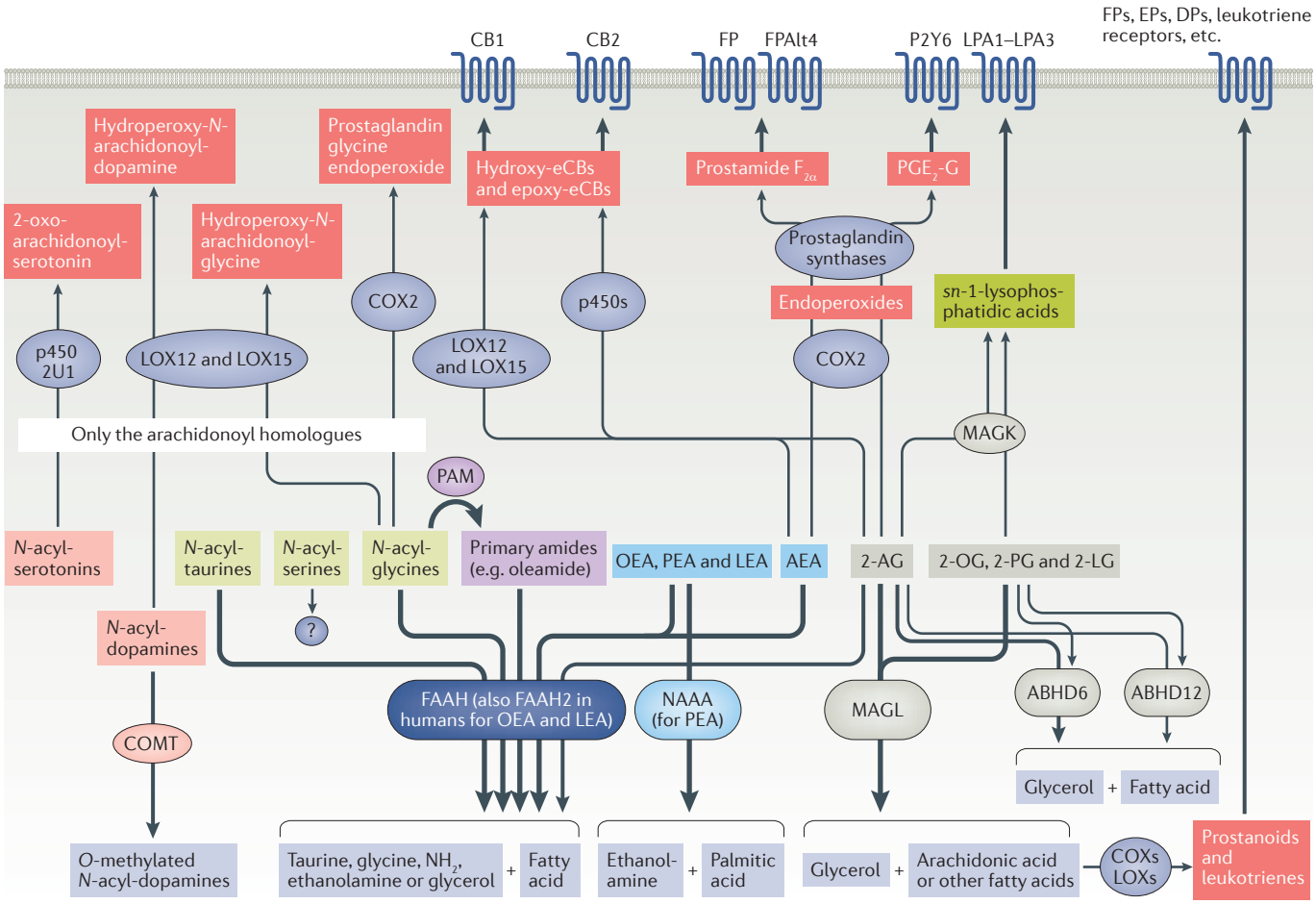

Figure 3 | **Catabolism of the endocannabinoidome mediators.** Oxidizing enzymes (indicated in medium blue) and more pathway-specific serine hydrolases are primarily responsible for the catabolism of endocannabinoidome signalling molecules. Fatty acid amide hydrolase 1 (FAAH) and monoacylglycerol lipase (MAGL) are thought to be the primary enzymes responsible for the catabolism of *N*-arachidonoyl-ethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) respectively. Products of oxidation (indicated in red) often signal through G protein-coupled receptors (GPCRs), as shown. The thickness of the arrows indicates the importance of the pathway in metabolite degradation. *N*-Acylethanolamines (NAEs) are shown in light blue, 2-acylglycerols (2-AcGs) are shown in grey, lipoamino acids are shown in light green, and the acyl neurotransmitters are shown in pink. FAAH (dark blue) catabolizes multiple endocannabinoidome signalling molecules. The receptor for prostamide $F_{2\alpha}$ has been suggested to be a heterodimer between the prostaglandin $F_{2\alpha}$ receptor (FP) and its splicing variant 4 (FPAlt4)[239]. Several NAEs, including AEA, as well as 2-AG have also been suggested to have non-specific intracellular carriers (not shown in the figure) such as fatty-acid-binding proteins[240] and heat shock 70 kDa protein (HSP70) (REF.[241]), which seem to be important for their inactivation. 2-LG, 2-linoleoyl glycerol; 2-OG, 2-oleoyl glycerol; 2-PG, 2-palmitoyl glycerol; ABHD, α/β-hydrolase; CB, cannabinoid receptor; COMT, catechol-*O*-methyltransferase; COX, cyclooxygenase; DPs, prostaglandin D_2 receptors; eCBs, endocannabinoids; EPs, prostaglandin E_2 receptors; LEA, *N*-linoleoyl ethanolamine; LOX, arachidonate lipoxygenase; LPA1, lysophosphatidic acid receptor 1; MAGK, monoacylglycerol kinase; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; OEA, *N*-oleoylethanolamine; P2Y6, P2Y purinoceptor 6; p450 2U1, cytochrome p450 2U1; PAM, peptidyl-glycine α-amidating monooxygenase; PEA, *N*-palmitoylethanolamine; PGE_2-G, prostaglandin E_2-glycerol.

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 *N*-acyl-amides, such as the *N*-acyl-taurines and the primary amides (such as the sleep-inducing factor oleamide), which preferably modulate non-CB1 and non-CB2 receptors[16,80,81]. MAGL is not selective for 2-AG over other 2-AcGs or even other monoacylglycerols[82]; other esterases, such α/β-hydrolase 6 (ABHD6) and ABHD12, which have been suggested to catalyse the hydrolysis of 2-AcGs, are likewise non-selective[83]. Oxidation of endocannabinoids occurs via the arachidonate cascade, which includes cyclooxygenase 2 (COX2; also known as PTGS2)[84], 12- and 15-lipoxygenases[85], and some cytochrome p450 oxygenases[86]. NAEs and 2-AcGs with saturated acyl chains are less likely to be metabolized through oxidation, whereas those with monounsaturated and polyunsaturated chains can be recognized by some of these enzymes.

Several different chemotypes of inhibitors of FAAH have been developed[32,33]. At least four of them, PF-04457845, BIA 10–2474, V158866 (ClinicalTrials.gov identifier NCT01748695) and JNJ-42165279 (NCT02432703 and NCT02498392), have been administered to humans, and a fifth, URB597, is about to be tested in patients with schizophrenia (NCT00916201). PF-04457845 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 that are yet to be fully established[35] but are 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 PF-04457845 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 entered phase I clinical trials. Although promising results were announced for ABX-1431 (see Related links), 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] 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 pause in 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 other than CB1 and CB2. Indeed, treatment of mice or humans with FAAH inhibitors increases the levels of AEA as well as OEA and PEA[36,89] and often unmasks effects mediated by peroxisome proliferator-activated receptor-α (PPARα) or TRPV1, for which OEA and PEA also 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 antagonized 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.

Other 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 catabolized to, other lipid mediators. A particular species of lysophosphatidic acid (LPA), *sn*-1-lyso-2-arachidonoyl-phosphatidic acid, which preferentially activates the GPCRs LPA receptor 2 (LPA2), 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 COX2, and the ensuing endoperoxides are converted by prostaglandin synthases to prostaglandin ethanolamides (prostamides) and prostaglandin 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 affect not only 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 DGLs reduces levels of 2-AG, as well as prostanoid and LPA signalling downstream of 2-AG[105], but increases DAG and protein kinase C signalling. Inhibition of NAPE-PLD, the enzyme that catalyses the direct conversion of NAPEs into NAEs[14], reduces AEA signalling at cannabinoid receptors and TRPV1, as well as NAE signalling at PPARα, but will also reduce the levels of the other bioactive product of the hydrolytic reaction, phosphatidic acid. Levels of NAPE-PLD substrates, which have been shown to affect metabolism, are also increased[106]. Finally, inhibition (or genetic inactivation) of FAAH or MAGL increases the levels of AEA or 2-AG, respectively, but may also increase their oxidation by COX2 and hence enhance prostamide and PG-G signalling[102,107]. For MAGL, this metabolic shift may also reduce prostanoid[98,99] and increase LPA[105] receptor activity.

In summary, the above considerations suggest 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, generalized knockout of the major endocannabinoid hydrolysing enzymes is accompanied by phenotypes that are sensitive to cannabinoid receptor antagonism (for example, see REF.[108]). These phenotypes are therefore due to elevation of endocannabinoid levels in tissues, although MAGL genetic inactivation can also produce CB1 desensitization owing to the subsequent elevation of levels of 2-AG, which functions as a CB1 agonist [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 knockouts of the corresponding genes or their mRNA transcripts might cast light on which enzyme needs to be inhibited, and when and where it needs to be inhibited, to selectively affect the levels of particular endocannabinoids and specifically modulate the activity of one or more endocannabinoid receptor. Such studies will need to include

not only the observation of the ensuing phenotypes but also 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 which lipid mediators are affected and to what extent they are affected can one surmise what type of receptors are modulated by the genetic (or pharmacological) manipulation of endocannabinoid anabolic and catabolic enzymes.

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. Examples include the *N*-acyl-amino acids (or lipoamino acids), such as the *N*-acyl-glycines, which are inactivated by FAAH and can activate the orphan GPCR GPR18 and, like AEA, can inhibit T-type Ca^{2+} channels (Ca_v3s)[58,111–114], and 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]. Examples of acyl neurotransmitters include the *N*-acyl-dopamines, most of which activate TRPV1 and inhibit FAAH and Ca_v3s[114,117–121], and the *N*-acyl-serotonins[122], which inhibit FAAH and often antagonize TRPV1 but also inhibit Ca_v3s and whose biosynthesis so far has been investigated in only *Drosophila melanogaster*[123–126]. Furthermore, other long-chain *N*-acyl-amides, such as *N*-arachidonoyl-tyrosine and *N*-arachidonoyl-tyramine, *N*-acyl-GABAs, *N*-acyl-alanines, *N*-acyl-methionines, *N*-acyl-prolines, *N*-acyl-valines, *N*-acyl-leucines, *N*-acyl-isoleucines and *N*-acyl-phenylalanines, have been identified[66,127,128], although their metabolism and/or biological effects have not yet been 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 may have numerous chemical features in common with the endocannabinoids, such as inactivating enzymes and molecular targets (FIGS. 1,2,3), the name '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*-1-lyso-2-arachidonoyl-LPA) and their biosynthetic precursors (NAPEs and 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 DGLs 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[133,134]. LPA1–LPA3, 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]. PPARα, 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-adipogenic 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? Second, do specific profiles for these hundreds of mediators exist for different individuals or distinct diseases, and will deciphering these signatures lead to new personalized diagnoses and treatments?

Multi-target drugs

On the basis of 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 problematic. One way to deal with endocannabinoid target promiscuity and metabolic enzyme redundancy is to develop drugs with multiple but specific groups of targets. For example, 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. Therefore, new compounds containing one pharmacophore that inhibits FAAH or MAGL and another that antagonizes 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 non-pungent TRPV1 agonist, was originally developed (but never marketed) as an analgesic, as it could rapidly desensitize TRPV1 and was later found to also inhibit endocannabinoid transport across the cell membrane[144]. This as-yet uncharacterized mechanism for the cellular reuptake of endocannabinoids is necessary for their degradation by intracellular catabolic enzymes[145,146]. Conversely, AM404, which was 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

Table 2 | **Selected marketed[a] and preclinical drugs with multiple therapeutically relevant endocannabinoidome targets**

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 (–) • eCB transport across the membrane (––)	• TRPV1 (++) • TRPA1 (++) • COX2 (–; ns and nss)	Pain and fever	147–152,253
Dipyrone (via its AM404-like metabolites)[a]	• CB1 (+) • CB2 (+)	• TRPV1 (+) • COX2 (–; ns and nss)	Pain and fever	254
ARN2508	FAAH (–––)	COX2 (–––; ns and nss)	Pain without gastric mucosal damage and IBDs	171
OMDM-198	FAAH (–)	TRPV1 (––)	Inflammatory pain and anxiety	158,159
OMDM-202	FAAH (–)	TRPA1 (++)	Inflammatory pain	158
AGN211335 and AGN211336	FAAH (––)	Heterodimer between FP and FPAlt4 (––)	Inflammatory pain	163
(*R*)-2-(2-fluorobiphenyl-4-yl)-*N*-(3-methylpyridin-2-yl) propanamide	FAAH (–)	COX2 (–; ns and ss)	Inflammatory pain and anxiety	169
Compound 11e	• FAAH (–) • eCB transport across the membrane (–)	COX2 (–; ss)	Inflammatory pain and anxiety	170
Compound 11f	• CB2 (+) • eCB transport across the membrane (–)	COX2 (–; ss)	Inflammatory pain and anxiety	170
AGN220653	FAAH (––)	Various prostanoid receptors (DP1, DP2, EP1, EP4, FPs and TP) (––)	Inflammatory pain	172
Compound 2 and compound 3	CB1 (––)	PPARα	Metabolic syndrome and hepatosteatosis	173
JZL195	• FAAH (––) • MAGL (–––)	None	Neuropathic and visceral pain, nausea, pruritus and depression (but not anxiety disorders)	88,174–178

The strength of the activation (+) or inhibition (–) is indicated by the number of + or –. CB, cannabinoid receptor; COX, cyclooxygenase; DP, prostaglandin D_2 receptor; eCB, endocannabinoid; EP, prostaglandin E_2 receptor; FAAH, fatty acid amide hydrolase 1; FP, prostaglandin F_2 receptor; FPAlt4, splicing variant 4 of FP; IBDs, inflammatory bowel disorders; MAGL, monoacylglycerol lipase; ns, non-selective for COX2 over COX1 (also known as PTGS1); nss, non-substrate-selective for inhibition of oxidation of 2-arachidonoylglycerol (2-AG) over arachidonic acid; PPARα, peroxisome proliferator-activated receptor-α; ss, substrate selective for inhibition of oxidation of 2-AG over arachidonic acid; TP, thromboxane receptor; TRPA1, transient receptor potential cation channel subfamily A member 1; TRPV1, transient receptor potential cation channel subfamily V member 1. [a]Lists drugs that have been tested in clinical trials and are currently marketed.

(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 and activation or desensitization of TRPV1[150–152]. *N*-Arachidonoyl-serotonin, initially designed as an FAAH inhibitor[123] and later shown to be an endogenous lipid[122], was found to also antagonize TRPV1 (REF.[124]) and to have CB1-mediated and TRPV1-mediated analgesic, anxiolytic and antidepressant activity in rodents[124,153–156]. 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 (REF.[158]), was shown to effectively reduce osteoarthritic pain in rats[159]. These compounds can counteract one of the aforementioned consequences of FAAH inhibition — the indirect activation of TRPV1, which not only exacerbates pain but also has 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 COX2 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 suggested for some synthetic prototypes of such compounds[163–166]. Interestingly, some previously developed COX inhibitors, the *R*-profens, were shown to inhibit the COX2-mediated peroxidation of endocannabinoids selectively over the peroxidation of arachidonic acid[167,168], 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[169]. Functionalization of β-caryophyllene, a naturally occurring CB2 agonist, generated an FAAH-specific and endocannabinoid substrate-specific COX2 inhibitor[170]. Adding a COX-blocking moiety to FAAH inhibitors might improve the analgesic efficacy of FAAH blockade in humans, and notably, inserting an 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 prostaglandin E_2

receptors (EPs), prostaglandin F_2 receptors (FPs) and prostaglandin D_2 receptors (DPs) and indirect activation of CB1 (REF.[172]).

Stronger efficacy can be obtained by not only 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 nanomolar 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, at least for some indications (TABLE 2). They may be used at lower doses than single MAGL inhibitors, thereby potentially minimizing the induction of drug tolerance and their addictive potential[174–178].

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.

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 standardized 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 neuropathic pain (only in Canada) or spasticity (in 30 countries so far) in patients with multiple sclerosis[21,179]. Preclinical studies with various combinations of THC and CBD have had positive outcomes[180–182]. In the clinic, nabiximols has been tested for the treatment of cancer pain, with mixed but promising results[183,184]; the progression of glioblastoma after recurrence of the tumour, with a seemingly positive outcome (see Related links); and the main symptoms of Huntington 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 end point 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 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 (see REF.[191] for review), but was rather due to a combination of effects on other targets, such as adenosine receptor A_{2a} and 5-hydroxytryptamine receptor 1A (5-HT_{1A})[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 of up to 800 mg per 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 aetiopathological 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 CBD (Epidiolex was recently approved by the FDA 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) (see REF.[201] for a recent review). For example, as already mentioned, phytocannabinoids, particularly CBD, CBG and THCV, activate and desensitize TRPV1 much in the same way as NAEs, *N*-acyl-taurines, *N*-acyl-dopamines and other *N*-acyl-amides do. CBD was reported to antagonize GPR55, which was suggested 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, which could begin with the chemical structures of multi-target phytocannabinoids and endocannabinoidome mediators. This could be especially useful when phytocannabinoids and endocannabinoidome mediators cannot be clinically developed because of stability, pharmacokinetics or marketing issues.

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

Phytocannabinoid	Effect on CB1 and CB2	Effect on TRP channels	Effect on PPARs and orphan GPCRs	Effect on enzymes and transporters	Effect on neurotransmitter receptors and voltage-dependent ion channels	Potential therapeutic uses	Refs
CBD	Negative allosteric modulator for CB1[a]	• TRPV1 (+) • TRPV2 (+) • TRPV3 (+)[a] • TRPA1 (+) • TRPM8 (–)	• PPARγ (+) • GPR55 (–) • GPR3 (–)[a] • GPR6 (–)[a] • GPR12 (–)[a]	• FAAH (–) • ENT (–) • eCB transport across the membrane (–)	• 5-HT_{1A} (+) • Glycine receptors (+) • $GABA_AR$ (+)[a] • Ca_V3s (–) • Ca_V1s (–) • $Na_V1.6$ (–)[a] • VDAC1 (–)[a]	Chronic and inflammatory pain, epilepsy, IBDs, schizophrenia, cancer and neuroinflammatory diseases	64,67,68,199, 202,255–260
CBDV	None	• TRPV1 (+) • TRPA1 (+) • TRPM8 (–)	None	• DGLα (–)[a] • eCB transport across the membrane (–)[a]	None	Epilepsy	67,68,199,256
CBDA	None	None	PPARγ (+)	• DGLα (–)[a] • NAAA (–)[a]	Positive allosteric modulator for 5-HT_{1A}[a]	Nausea and cancer	199,202,256, 261,262
THCV	• CB1 (–) • CB2 (+)	• TRPV1 (+) • TRPV2 (+) • TRPV3 (+) • TRPA1 (+)[a] • TRPM8 (–)	None	None	5-HT_{1A} (+)[a]	Obesity, metabolic syndrome, insulin resistance, steatosis, schizophrenia and inflammatory pain	67,199,256, 257,263
CBG	Weak CB2 agonist	• TRPV1 (+) • TRPV2 (+) • TRPA1 (+) • TRPM8 (–)	PPARγ (+)	eCB transport across the membrane (–)[a]	• ADRA2 (+)[a] • 5-HT_{1A} (–)[a]	Cancer, neurodegenerative diseases and IBDs	67,202,256, 257,261,264
CBC	None	TRPA1 (+)	None	• ENT (–)[a,b] • eCB transport across the membrane (–)[a]	None	Pain and gliosis	67,199, 256,260
THCA	None	None	PPARγ (+)	• DGLα (–)[a] • MAGL (–)[a]	None	Neurodegenerative diseases	67,256,261

+ indicates activation, and – indicates inhibition. 5-HT_{1A}, 5-hydroxytryptamine receptor 1A; ADRA2, α_2-adrenergic receptor; Ca_V1s, L-type Ca^{2+} channels; Ca_V3s, T-type Ca^{2+} channels; CBC, cannabichromene; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; CBG, cannabigerol; DGLα, *sn*-1-specific diacylglycerol lipase-α; $GABA_AR$, $GABA_A$ receptor; eCB, endocannabinoid; ENT, equilibrative nucleoside transporter; FAAH, fatty acid amide hydrolase 1; GPCRs, G protein-coupled receptors; GPR3, GPCR 3; IBDs, inflammatory bowel disorders; MAGL, monoacylglycerol lipase; NAAA, *N*-acylethanolamine-hydrolysing acid amidase; $Na_V1.6$, voltage-gated sodium channel type 1.6 (also known as SCN8A); PPAR, peroxisome proliferator-activated receptor; THCA, Δ^9-tetrahydrocannabinolic acid; THCV, Δ^9-tetrahydrocannabivarin; TRP, transient receptor potential; TRPA1, transient receptor potential cation channel subfamily A member 1; TRPM8, transient receptor potential cation channel subfamily M member 8; TRPV1, transient receptor potential cation channel subfamily V member 1; VDAC1, voltage-dependent anion-selective channel protein 1. [a]On the basis of only one study; [b]On the basis of only in vivo pharmacological data.

Synthetic cannabinoids

Whereas plant cannabinoids, either as purified compounds (CBD) or as standardized botanical extracts (nabiximols), are achieving success in the clinic, synthetic cannabinoids with ultra-potent agonist activity at CB1 have started obtaining a different type of fame. THC is a relatively high affinity but inefficacious agonist for both CB1 and CB2, even compared with 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 fivefold 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 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 fairly simple organic reactions, are becoming a very serious problem because of their often dangerous side effects, which range from agitated delirium, hallucinations, tachycardia and hypertension to psychosis, seizures, lethargy and coma[208,209]; these effects also occasionally lead 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 the deaths had no other discernible cause on autopsy, and the autopsies of these five deaths uncovered detectable plasma levels of synthetic CB1 agonists such as PB-22 (1.1 ng per ml), JWH-210 (12.0 ng per ml), XLR-11 (1.3 ng per ml), AB-CHMINACA (8.2 ng per ml), UR-144 (12.3 ng per ml) and JWH-022 (3.0 ng per ml)[210]. Such levels

may yield concentrations from ~2 to 20 nM, which, for these compounds, would be sufficient to fully activate CB1.

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 have used these compounds[210]. New 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 their potential dangerous side effects 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 administered with relative overall safety to thousands of healthy volunteers and patients with obesity in clinical trials[26,29], might block the acute effects of synthetic cannabinoids[211] if their consumption can be deemed responsible for life-threatening effects in hospitalized individuals. By contrast, 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.

Endocannabinoids and gut microbiota

The gut microbiota is increasingly known to be involved in a plethora of physio-pathological conditions (see REF.[212] for a recent review). Interestingly, a probiotic, *Lactobacillus acidophilus*, was shown to produce antinociceptive effects against visceral pain by increasing the expression of CB2 and μ-type opioid receptor (MOR1) 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 (REF.[214]). Accordingly, in a subsequent study, the protective effects of capsaicin, the prototypical TRPV1 agonist and a 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[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 on metabolism.

The relationship between the endocannabinoid system and the gut microbiota seems to be bi-directional. Blockade of CB1 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[216], and subsequently reducing inflammation[217]. These data indicate that the increased 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 suggest the existence of a vicious cycle between increased 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 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 dyslipidaemia[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 resulting increased gut permeability) and hence could be partially transferred to wild-type germ-free mice[221]. These data are counter-intuitive, 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 had 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 (that is, GPR119, TRPV1 and PPARα) other than CB1. This is the first study to suggest an important role for some non-endocannabinoid lipid mediators of the endocannabinoidome in inhibiting 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 endocannabinoi-dome mediator is affected and where these effects occur.

An intestine-specific knockout of myeloid differentiation primary response protein (MYD88) also linked intestinal inflammation with the endocannabi-noidome following the quantification of metabolites. MYD88 is a central adaptor molecule for most of the Toll-like receptors through which microbiota-derived inflammatory molecules (such as LPS) signal to the immune system. MYD88 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, and had 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-AcGs (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-glycerol 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 are partly responsible for some of these effects in mice[225]. Perturbation of the microbiota by prolonged treatment with antibiotics was accompanied by an inflammatory state and strong behavioural changes in two experimental tests of depression. The authors also observed altered brain-derived neurotrophic factor (BDNF)–tropomyosin-related kinase B (TRKB; also known as NTRK2) 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, 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.

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 the market, is increasingly suggested to act in part through the endocannabinoidome; the *R*-profens may owe part of their analgesic effects to reduced COX2-mediated peroxidation of endocannabinoids; and ketamine may also act partly through these mediators[230].

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 other disorders[231]. All this should not come as a surprise, as several successful therapeutic drugs that have been on the market for years (such as many nonsteroidal anti-inflammatory drugs, tyrosine kinase inhibitors and metformin) are now known to be promiscuous in their mechanism of action, and polypharmacology is increasingly used to treat several multi-factorial disorders in a more efficacious and safer manner.

Therefore, there is a strong rationale for developing synthetic or natural multi-target drugs from the endocannabinoidome. However, efforts will also need to be dedicated to other strategies, such as the clinical development of some of the over 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 individuals who are obese (the population in which rimonabant was unsafe) or for orphan and otherwise untreatable diseases. CB1 antagonists could be useful in treating muscular dystrophies, for example, given the anti-differentiating activity of CB1 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[48,49]. Exploiting the positive and negative allosteric sites in CB1 and CB2 (REFS[37-39]) could be useful in diseases such as chronic pain, cancer, anxiety, depression and schizophrenia and in metabolic and neuroinflammatory disorders. In this respect, recently identified endogenous negative allosteric modulators of cannabinoid receptors, like some hemopressin 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 endocannabinoid-like 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, 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 that these tools are now proposed to be used for precision and personalized 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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