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Targeting Endocannabinoid Signaling: FAAH and MAG Lipase Inhibitors

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THC, endocannabinoids, FAAH, MAGL, cannabinoid receptor

Abstract

Inspired by the medicinal properties of the plant *Cannabis sativa* and its principal component (−)-*trans*-Δ^9-tetrahydrocannabinol (THC), researchers have developed a variety of compounds to modulate the endocannabinoid system in the human brain. Inhibitors of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), which are the enzymes responsible for the inactivation of the endogenous cannabinoids anandamide and 2-arachidonoylglycerol, respectively, may exert therapeutic effects without inducing the adverse side effects associated with direct cannabinoid CB_1 receptor stimulation by THC. Here we review the FAAH and MAGL inhibitors that have reached clinical trials, discuss potential caveats, and provide an outlook on where the field is headed.

INTRODUCTION

THC: (−)-*trans*-Δ^9-tetrahydrocannabinol

$CB_{1/2}$ receptor: cannabinoid 1/2 receptor

AEA: *N*-arachidonoylethanolamine (anandamide)

2-AG: 2-arachidonoylglycerol

ECS: endocannabinoid system

FAAH: fatty acid amide hydrolase

MAGL: monoacylglycerol lipase

MS: multiple sclerosis

CBD: cannabidiol

The plant *Cannabis sativa* has been used for medicinal and recreational purposes for centuries. It contains over 500 compounds, of which around 100 belong to the class of cannabinoids (1). In the 1960s, the main psychoactive component, (−)-*trans*-Δ^9-tetrahydrocannabinol (THC), was isolated and characterized (2). The cannabinoid 1 (CB_1) and 2 (CB_2) receptors, the molecular entities by which THC exerts its characteristic effects, were identified three decades after the structure of THC was determined (3, 4). This discovery started the search for the endogenous ligands that bind to these receptors (so-called endocannabinoids). *N*-arachidonoylethanolamine (anandamide or AEA) was discovered as the first endocannabinoid and was followed shortly by 2-arachidonoylglycerol (2-AG) (5, 6), which prompted the investigation of their biosynthesis, metabolism, transport, and physiological roles (7). Together, the $CB_{1/2}$ receptors, endocannabinoids, and the proteins responsible for their biosynthesis and inactivation constitute the endocannabinoid system (ECS). Here we briefly discuss the potential therapeutic and adverse effects of medical cannabis and review potential alternative strategies that are being considered based on modulation of the ECS with a focus on experimental drugs that target fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the enzymes that inactivate endocannabinoids (8, 9).

CANNABIS SATIVA: A VERSATILE THERAPEUTIC PLANT?

Cannabis has been exploited as a medical remedy in various cultures, and many anecdotal stories about its therapeutic properties have accumulated over centuries (10). More recently, clinical trials were conducted to evaluate the efficacy and safety of cannabis and THC for several diseases, but convincing evidence for most indications is still lacking (11, 12).

Therapeutic Use of Cannabis-Based Products

THC has antiemetic effects comparable or superior to standard antiemetics, although statistical significance was not always reached in clinical trials (13, 14). Marinol (dronabinol, synthetic THC) and Cesamet (nabilone, a synthetic analog of THC) are approved for the suppression of nausea and vomiting in patients receiving chemotherapy. Cannabis also stimulates appetite, a response that is exploited in the treatment of weight loss and lack of appetite in patients with HIV/AIDS, cancer, and Alzheimer's disease (15–17).

Multiple sclerosis (MS) is a chronic disease associated with a variety of distressing symptoms, including pain, muscle spasms, and fatigue (18). Cannabis is claimed to alleviate many of these symptoms (19). However, objective assessment of symptom relief is difficult, which has complicated the study of various cannabis formulations (20). Sativex (nabiximols), a cannabis extract of THC and cannabidiol (CBD) (1:1), is approved in Canada and several European countries for symptom relief in MS. Relying on self-reported accounts, a large-scale trial reported that Sativex improved spasticity and sleep disturbance, but evidence for significant and clinically relevant efficacy from objective end points is lacking (21).

Despite the widespread use of cannabis for pain relief, clinical evidence in support of this is surprisingly limited (22, 23). Overall, cannabis appears to be effective in chronic neuropathic pain, whereas it is ineffective in acute pain (24, 25). Beneficial effects have been observed in chronic neuropathic pain both related and unrelated to MS (26) and in inflammatory pain (27, 28). In addition, cannabis-based medicines have been associated with positive results in Tourette's syndrome (29, 30) and in levodopa-induced dyskinesia in Parkinson's disease (31). However, large-scale, randomized, placebo-controlled, double-blind clinical trials have not been reported (12).

The second most abundant cannabinoid in *C. sativa* is non-psychotropic CBD (1), and its mechanism of action is not fully understood (32). It has been suggested that CBD has therapeutic potential by itself (33), and a number of clinical trials have been completed or are ongoing to investigate the safety and efficacy of CBD in epilepsy (34). These efforts have resulted in the US Food and Drug Administration approval of Epidiolex, a formulation of CBD, for the treatment of seizures in rare forms of pediatric epilepsy (35). Furthermore, CBD has recently gained increased attention as a novel therapeutic for the treatment of anxiety and sleep disorders. However, no convincing evidence from large, well-controlled clinical trials exists for its efficacy in these indications (36).

Limitations of Cannabis-Based Therapeutics

The medicinal effects of cannabis occur alongside its potential to induce (adverse) side effects, which include euphoria, being high, anxiety, acute psychosis, panic, impaired memory and motor coordination, and induction of schizophrenia in genetically predisposed individuals (20) (**Figure 1**). Prolonged use may lead to impaired cognitive performance (37). The use of cannabis is also associated with adverse cardiovascular events, e.g., myocardial infarction, coronary thrombosis, and stroke (38).

Apart from the adverse effects that limit the widespread use of THC as a therapeutic agent, dosing and administration options are not optimal. Oral administration is possible, but cannabinoids are sequestered into fat and only slowly released into the plasma. A pronounced first-pass effect contributes to variable concentrations of active THC in the blood. As a result, the therapeutic window is narrow and reliable dosing is difficult (39). Therapeutic effects are more pronounced

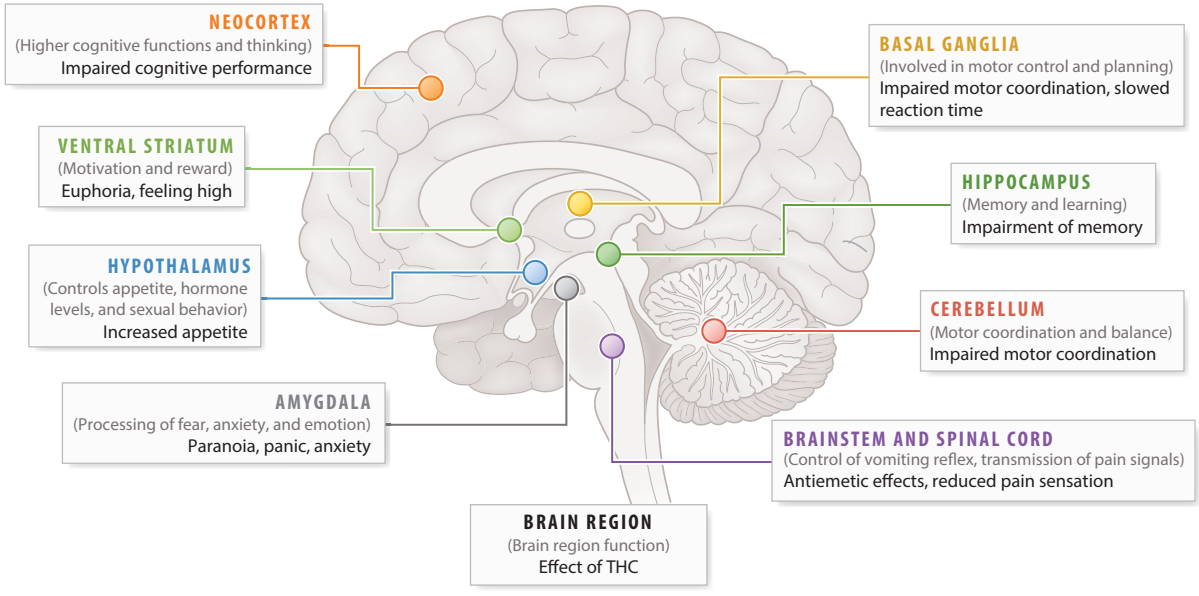


Figure 1

Effects of (−)-*trans*-Δ^9-tetrahydrocannabinol (THC) on the human brain. THC, the main active constituent of cannabis, binds to and activates the cannabinoid CB_1 receptor throughout the brain. Depending on the regional distribution and abundancy of CB_1 receptors in the brain and the physiological function of the different brain regions, THC exerts different effects. Some effects have been exploited for therapeutic purposes, whereas others represent adverse effects that limit the widespread use of medicinal cannabis. Figure adapted from the National Institute on Drug Abuse (**https://www.drugabuse.gov/publications/research-reports/marijuana**).

if cannabis is smoked and inhaled, but the adverse effects of smoke inhalation and the fear of abuse limit the utility of this form of administration (20).

GABA: γ-aminobutyric acid

Another drawback of medicinal cannabis as a therapeutic strategy is that prolonged usage may lead to tolerance. Mechanisms contributing to tolerance are downregulation of CB_1 receptor expression, receptor internalization, and receptor desensitization (40). Cannabis tolerance underlies physical dependence on cannabis. Sudden discontinuation of long-term cannabis use may lead to withdrawal symptoms that include irritability and loss of sleep and appetite (41).

The limitations of direct CB_1 receptor activation are further demonstrated by the effects of potent synthetic, full agonists of the CB_1 receptor (42). These types of compounds have been misappropriated in so-called herbal mixtures as designer drugs, which are known as K2 or spice (43). They often contain a blend of synthetic CB_1 receptor agonists and are associated with increased cardiovascular risks, which can be fatal (38).

THE ENDOCANNABINOID SYSTEM: AN INSPIRATION FOR MODERN DRUG DISCOVERY

Since both the therapeutic and adverse side effects of THC mainly result from CB_1 receptor activation, separation of the desired from untoward effects may be difficult (44). The discovery of the ECS, however, provided new opportunities to investigate alternative therapeutic strategies. Next, we briefly describe the ECS as a background for the clinical development of FAAH and MAGL inhibitors. Other excellent reviews provide a more detailed account of the ECS (45–47).

Cannabinoid CB_1 and CB_2 Receptors

The central nervous system (CNS) effects of THC result from the widespread activation of the CB_1 receptor in the brain, where it is the most abundant G protein–coupled receptor. Brain regions of high expression include the hippocampus, cerebellum, basal ganglia, and cerebral cortex (48), which is in accordance with the adverse effects of cannabis such as impairment in short-term memory, motor coordination, and cognitive functions (44) (**Figure 1**). Studies in the forebrain found that the CB_1 receptor is predominantly located on the plasma membrane of presynaptic GABAergic interneurons (49). In addition to its widespread expression on GABAergic terminals, the CB_1 receptor is also found on axon terminals of glutamatergic neurons throughout the brain (50). Intracellularly, the CB_1 receptor can localize to mitochondria and thereby regulate neuronal energy metabolism (51). Low expression of the CB_1 receptor in astrocytes, oligodendrocytes, and microglia further contributes to the modulation of synaptic function (52). The CB_1 receptor regulates various behaviors, including sleep, fear and stress responses, learning, memory, and food intake, through changes in gene expression and modulation of synaptic plasticity (53). The CB_1 receptor is also expressed in the periphery (54, 55). In the gastrointestinal tract, the CB_1 receptor is expressed under physiological conditions in nonneuronal cells and cells of the enteric nervous system and contributes to the regulation of food intake and energy balance (56). In other tissues, e.g., liver and cardiovascular system, CB_1 receptor expression is low under healthy conditions but upregulated in pathological states (55).

The CB_1 receptor is coupled to heterotrimeric $G_{i/0}$ proteins, which are stimulated upon receptor activation by synthetic agonists, cannabinoids, or endocannabinoids. Release of α and βγ subunits from the $G_{i/0}$ proteins leads to adenylyl cyclase inhibition, stimulation of the MAPK and PI3K/Akt pathways, and modulation of the activity of several types of ion channels (57). Inactivation of calcium influx through N-type Ca^{2+} channels into presynaptic cells suppresses neurotransmitter release, thereby modulating synaptic plasticity (45). Different active receptor

conformations lead to different signaling pathways (58); this can be exploited with allosteric CB_1 receptor modulators that have the potential to target the CB_1 receptor in a subtype- and pathway-specific manner, which may potentially limit CNS side effects (59).

The CB_2 receptor is mainly expressed on cells of the immune system but has also been found in the brain stem (60). The CB_2 receptor modulates several signaling pathways via $G_{i/0}$ proteins but does not change ion channel activities (61). CB_2 receptor expression is increased in pathological states (62) and implicated in inflammation and pain management. It is thus a possible target for pain relief and tissue injury (62, 63).

TRPV1: transient receptor potential vanilloid type-1

PPARγ: peroxisome proliferator-activated receptor γ

DAG: diacylglycerol

DAGLα/β: diacylglycerol lipase α/β

The Endocannabinoids

Both CB_1 and CB_2 receptors are activated by AEA (5) and 2-AG (6, 64), which are the best-studied endogenous ligands of the cannabinoid receptors. AEA is a high-affinity partial agonist of the CB_1 receptor, while it has low-affinity binding for the CB_2 receptor (65, 66). 2-AG is a full agonist at both receptors with higher intrinsic activity than AEA (67, 68). 2-AG acts as a retrograde messenger and causes depolarization-induced suppression of inhibition or excitation by inhibiting the release of GABA or glutamate, respectively, from presynaptic cells (69, 70) (**Figure 2**). While 2-AG appears to be the bona fide retrograde messenger, AEA can mediate long-term depression via retrograde signaling in certain brain areas (71). AEA is also a full agonist of the transient receptor potential vanilloid type-1 (TRPV1) ion channel (72), albeit with lower affinity than for the $CB_{1/2}$ receptors, and can activate the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (73).

Biosynthesis and inactivation of AEA and 2-AG contribute to the regulation of their signaling function and therefore present excellent targets for pharmacological intervention (**Figure 2**). Both endocannabinoids are produced from lipid precursors by different biosynthetic pathways in a Ca^{2+}-dependent manner (74) (**Figure 3**). The first step in AEA biosynthesis is the formation of the low-abundance phospholipid *N*-arachidonoylphosphatidylethanolamine (NArPE) by a calcium-dependent *N*-acyltransferase (CaNAT) (75, 76). Formation of AEA from NArPE is catalyzed by *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD) and also by other pathways not involving NAPE-PLD (77, 78). The main precursors for 2-AG formation are diacylglycerols (DAGs), which are formed by hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) by phospholipase C-β (PLC-β) and cleaved by DAG lipase α or β (DAGLα or β) to release 2-AG (79, 80). An alternative pathway involves the formation of lysophosphatidylinositol, which can be hydrolyzed to 2-AG (81). The postsynaptic location of DAGLα is in line with the proposed role of 2-AG as a retrograde neurotransmitter (82). Inhibitors of 2-AG, but not AEA, biosynthesis have been developed that are active in vivo and are useful tools to study the physiological roles of the ECS (83, 84).

Endocannabinoids need to reach their target receptors on the presynaptic cell membrane to exert their function. However, mechanisms of release, transport across the extracellular space, and uptake are not well understood and are debated (85, 86). Lipid-carrier proteins have been proposed for intracellular transport (87), and endocannabinoids have been found in extracellular vesicles, suggesting their involvement in release and cell-to-cell transport (88). Once the endocannabinoids have reached their destination and activated cannabinoid receptors on the target cell, they are taken up and degraded, thereby terminating the signaling event. Among the proposed mechanisms for cellular uptake is facilitated transport via an unknown membrane transporter (89). Although the mechanism of endocannabinoid uptake remains elusive, small molecules have been developed that specifically inhibit cellular uptake (90). After being taken up, endocannabinoids are shuttled to their site of degradation.

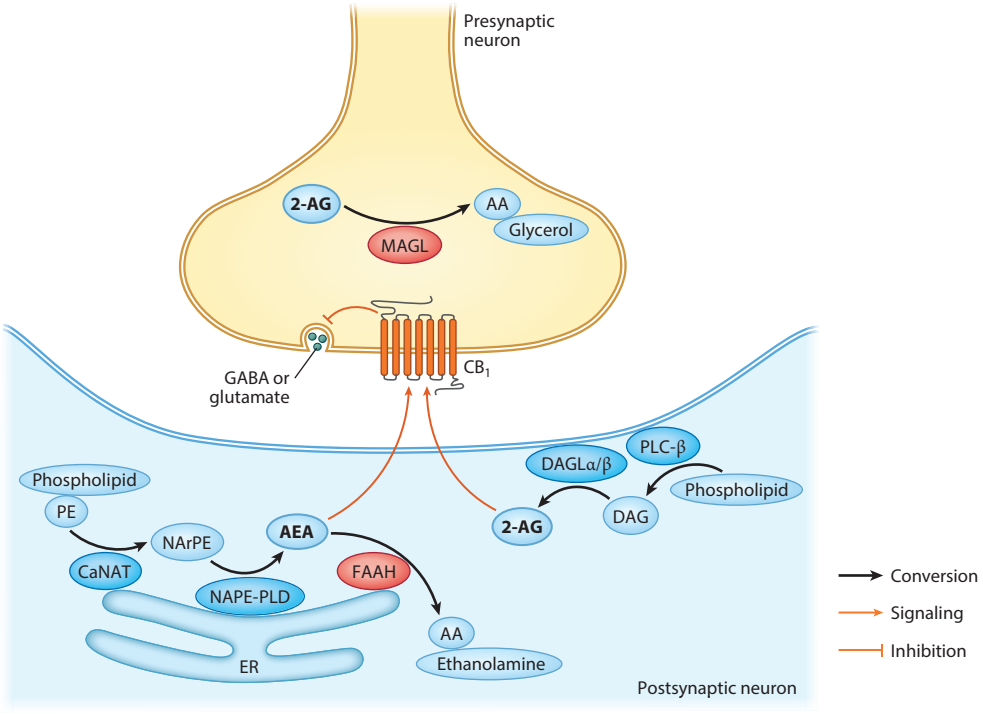


Figure 2

Endocannabinoid synthesis, degradation, and signaling function at the synapse. Sequential action of CaNAT and NAPE-PLD generates AEA. Both enzymes are found on intracellular membranes, but it is not clear whether they locate to pre- or postsynaptic neurons. Inactivation of AEA occurs mainly on intracellular membranes at postsynaptic sites by FAAH. PLC-β and DAGLα/β, the enzymes catalyzing 2-AG biosynthesis, associate with the plasma membrane. DAGLα/β is found on postsynaptic neurons, in contrast to the 2-AG-deactivating hydrolase MAGL, which localizes to the presynaptic neuron. AEA and 2-AG activate CB_1 receptors on the presynaptic plasma membrane. Mechanisms of release and extracellular transport, however, remain elusive. Enzymes involved in endocannabinoid synthesis are depicted in blue. Enzymes degrading 2-AG and anandamide are shown in red. Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, *N*-arachidonoylethanolamine (anandamide); CaNAT, calcium-dependent *N*-acyltransferase; CB_1, cannabinoid 1; DAG, diacylglycerol; DAGL, diacylglycerol lipase; ER, endoplasmic reticulum; FAAH, fatty acid amide hydrolase; GABA, γ-aminobutyric acid; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidylethanolamine phospholipase D; NArPE, *N*-arachidonoylphosphatidylethanolamine; PE, phosphatidylethanolamine; PLC-β, phospholipase C-β.

Endocannabinoid Inactivation

The main route for inactivation of AEA and 2-AG is their hydrolysis to arachidonic acid (AA) and ethanolamine or glycerol, respectively (**Figures 2** and **3**). Endocannabinoids can also undergo oxidative metabolism by lipoxygenases (91, 92), cyclooxygenases (93, 94), and cytochrome P450 (95), forming new molecules with potential physiological roles (96). FAAH is responsible for the hydrolysis of AEA (8), whereas the majority of 2-AG is hydrolyzed by MAGL (97, 98). Approximately 15% of brain 2-AG is hydrolyzed by α,β-hydrolase domain–containing proteins 6 and 12 (ABHD6 and ABHD12, respectively) (97). These enzymes belong to the family of serine hydrolases. FAAH has an unusual serine-serine-lysine catalytic triad (99), whereas MAGL, ABHD6, and ABHD12 have a serine-histidine-aspartate triad (98). FAAH has broad substrate selectivity toward fatty acid amides, including AEA and other *N*-acylethanolamines (NAEs), oleamide (8), and *N*-acyltaurines (100), while MAGL hydrolyzes monoacylglycerols. FAAH and MAGL are highly expressed in the CNS but can also be found in peripheral tissues such as kidney, lung, liver, gastrointestinal tract,

AA: arachidonic acid

ABHD6/12: α,β-hydrolase domain–containing protein 6/12

NAE: *N*-acylethanolamine

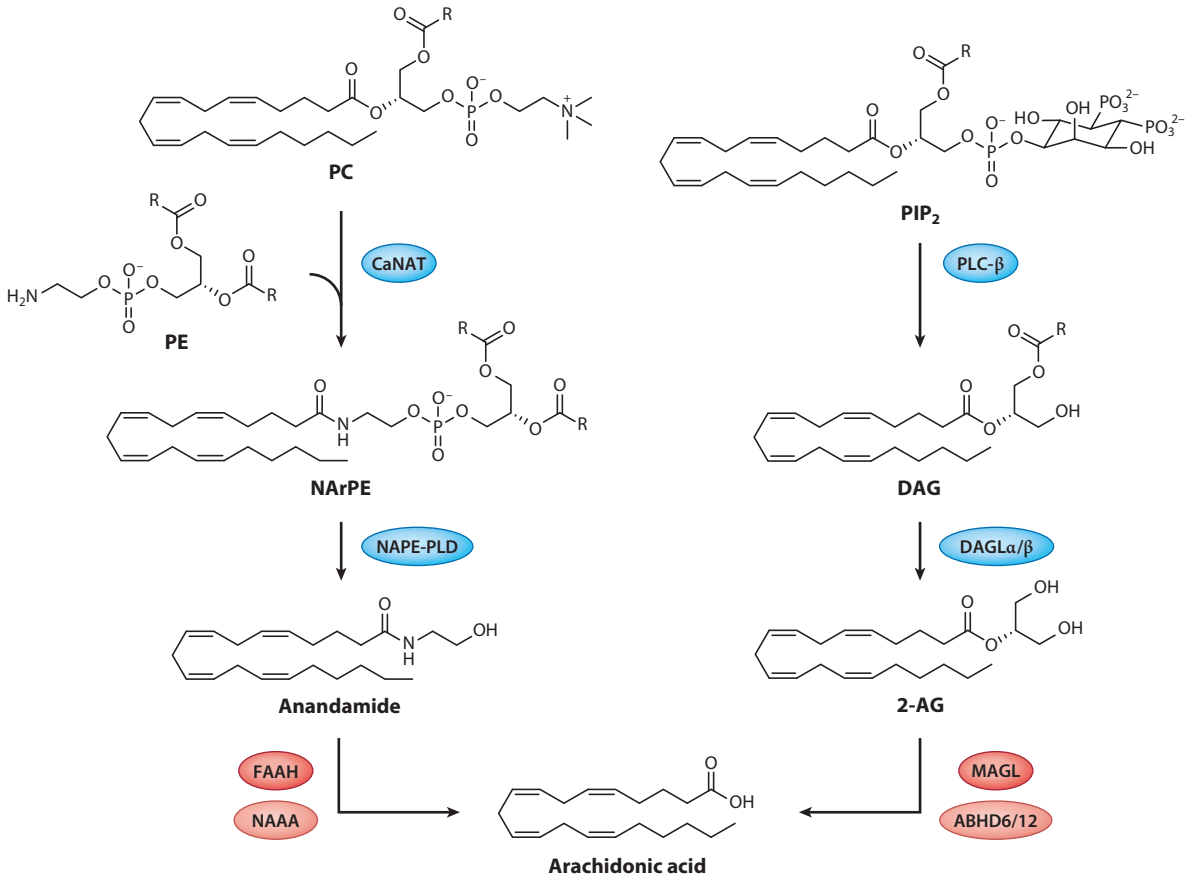


Figure 3

Major biosynthetic and metabolic pathways of AEA and 2-AG. Enzymes involved in endocannabinoid synthesis are depicted in blue. Enzymes degrading 2-AG and anandamide are shown in red, with FAAH and MAGL highlighted in dark red. Abbreviations: 2-AG, 2-arachidonoylglycerol; ABHD6/12, α/β-hydrolase domain–containing protein 6/12; AEA, anandamide; CaNAT, calcium-dependent *N*-acyltransferase; DAG, diacylglycerol; DAGLα/β, diacylglycerol lipase α/β; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAAA, *N*-acylethanolamine acid amide hydrolase; NArPE, *N*-arachidonoylphosphatidylethanolamine; NAPE-PLD, *N*-acylphosphatidylethanolamine phospholipase D; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC-β, phospholipase C-β.

urinary bladder, prostate, and testis (101–105). Within the CNS, FAAH activity is primarily found in principal neurons of the hippocampus, cerebellum, cerebral cortex, and olfactory bulb (101, 106, 107). FAAH localizes to intracellular membranes in postsynaptic neurons (108) (**Figure 2**).

Studies with FAAH knock-out (KO) mice and specific inhibitors have shown that elevation of AEA modulates anxiety and pain sensation without characteristic cannabinoid intoxication symptoms, e.g., catalepsy, reduced body temperature, or stimulated feeding (109–112). The irreversible FAAH inhibitor URB597 was tested extensively in vivo and was shown to have analgesic (113, 114), anxiolytic (112), and antidepressant (115) effects as well as a positive impact in models of epilepsy (116), schizophrenia (117), and post-traumatic stress disorder (PTSD) (118). In addition, inhibition of FAAH activity ameliorated the spasticity in chronic relapsing experimental allergic encephalomyelitis mice, a model of MS (119). Higher primates, including humans, express a second isoform, FAAH-2, with very low abundance in the brain (102). NAE-selective acid amidase,

which is mainly expressed in macrophages, is a third enzyme that participates to a lesser extent in AEA degradation (120).

ABPP: activity-based protein profiling

ADME: absorption, distribution, metabolism, excretion

MAGL exists in two tissue-specific splicing isoforms (121) and is associated with membranes of presynaptic neurons (97) (**Figure 2**). MAGL is found in the hippocampus and cerebellum and also in the anterior thalamus, where it is located in axon terminals of granule cells, CA3 pyramidal cells, and excitatory and inhibitory interneurons (9, 106, 107). Astrocytes and microglia express MAGL to a lesser extent (122, 123). Genetic inactivation of MAGL leads to a substantial reduction in brain 2-AG hydrolytic activity, accompanied by tenfold elevations in brain 2-AG levels and concomitant reduced AA levels (124). MAGL KO mice also have increased 2-AG concentrations in the thymus, spleen, and liver (123). Elevation of 2-AG levels in the brain produced by MAGL inhibition with the irreversible inhibitor JZL-184 is associated with anxiolytic (125) and antidepressant (126) effects as well as antinociception (127, 128) via the CB_1 receptor (122). Persistent elevation of 2-AG levels leads to desensitization and downregulation of the CB_1 receptor and tolerance to CB_1 receptor agonists (124, 129). Acute or chronic low dosing of a MAGL inhibitor, however, provides a therapeutic window in which CB_1 receptor–dependent antinociceptive effects are preserved without receptor desensitization (124). Moreover, blocking MAGL activity can exert anti-inflammatory and neuroprotective properties by preventing the downstream metabolism of AA to proinflammatory prostaglandins (130, 131). In that respect, MAGL inhibition may have the potential for treating neuropathic pain and neurodegenerative diseases accompanied by neuroinflammation such as Alzheimer's disease, Parkinson's disease, or MS (122, 131–133). Interestingly, only dual inhibition of FAAH and MAGL recapitulates the full spectrum of behavioral effects of CB_1 receptor activation by THC, demonstrating the collaborative nature of AEA and 2-AG signaling in the brain (134).

THE CLINICAL PROGRESS OF FAAH AND MAGL INHIBITORS

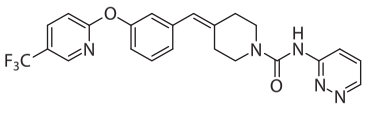
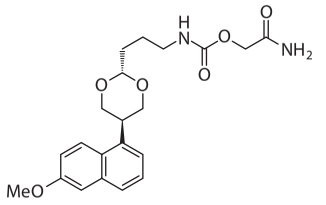
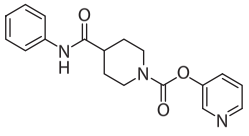
In light of the promising preclinical data with genetic and pharmacological inhibition of endocannabinoid hydrolysis, several pharmaceutical companies have initiated clinical trials with FAAH and MAGL inhibitors. In the sections below, we summarize the preclinical and clinical data obtained with these experimental drugs.

FAAH Inhibitors

The clinical investigation of FAAH inhibitors has progressed into the successful development of suitable experimental drugs by pharmaceutical companies Pfizer, Sanofi-Aventis, Astellas Pharma, Vernalis, and Janssen Pharmaceutica (135).

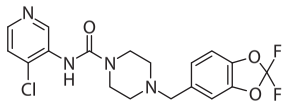
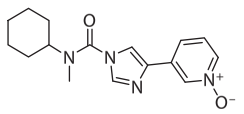
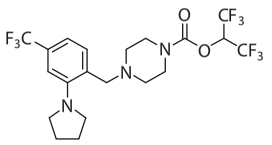
PF-04457845. Pfizer developed PF-04457845, the first FAAH inhibitor to reach clinical phase II trials. PF-04457845 is a covalent irreversible inhibitor that carbamoylates the enzyme's active site catalytic serine (Ser241) (136). In vitro profiling of PF-04457845 revealed a high potency for human FAAH (**Table 1**) and remarkable selectivity with respect to other members of the serine hydrolase superfamily, as determined by activity-based protein profiling (ABPP) (136, 137). Pharmacokinetic characterization in rats, dogs, and human in vitro assays revealed excellent absorption, distribution, metabolism, and excretion (ADME) properties and suitability for once-daily oral administration in humans. In rats, PF-04457845 [0.1 mg/kg per os (p.o.)] produced near-complete inhibition of FAAH (>98%) and a three-to-sevenfold increase of AEA levels in the plasma and brain.

Table 1 Drugs in clinical trials that inhibit FAAH or MAGL

Drug	Structure	IC_{50} (reference)	Clinical phase	Indication(s) (trial)	Efficacy/trial status (reference)
FAAH inhibitors					
PF-04457845		7 nM (136)	Phase II	Inflammatory pain in osteoarthritis (NCT00981357, 2009-014734-16)	Not efficacious (141)
				Cannabis withdrawal (NCT01618656)	Efficacious (140)
				Cannabis use disorder (NCT03386487)	Recruiting
				Fear extinction (2016-005013-47)	Ongoing
				Fear conditioning (NCT01665573)	Ongoing
SSR411298		63 nM[a] (144)	Phase II	Major depressive disorder (NCT00822744, 2008-001718-26)	Not efficacious (145)
				Cancer pain (NCT01439919, 2011-002557-56)	Terminated (146)
ASP8477		4 nM (147)	Phase II	Peripheral neuropathic pain (NCT02065349, 2013-002521-27)	Not efficacious (149)
V158866	Unknown	38 nM (150)	Phase II	Neuropathic pain (NCT01748695)	Not efficacious

(*Continued*)

Table 1 (*Continued*)

Drug	Structure	IC_{50} (reference)	Clinical phase	Indication(s) (trial)	Efficacy/trial status (reference)
JNJ-42165279		70 nM (153)	Phase II	Major depressive disorder (NCT02498392, 2015-002007-29) Social anxiety disorder (NCT02432703)	NA
				Autism (NCT03664232)	Recruiting
BIA 10-2474		7,500 nM (158)	Phase I	NA	Withdrawn (157)
MAGL inhibitors					
ABX-1431		14 nM (161)	Phase I	Functional dyspepsia (NCT02875678)	Terminated
				Hyperalgesia (NCT02929264) Central pain (NCT03138421) Neuropathic pain (NCT03447756)	NA
				Tourette syndrome (NCT03058562)	Efficacious (162)
			Phase II	Tourette syndrome and chronic motor tic disorder (NCT03625453, 2018-000100-41)	Ongoing

[a]In vitro mouse FAAH.

For the IC_{50} column, unless otherwise noted, values are for in vitro human FAAH. Abbreviations: FAAH, fatty acid amide hydrolase 1; IC_{50}, half maximal inhibitory concentration; MAGL, monoacylglycerols lipase; NA, not available.

PF-04457845 showed CB_1 and CB_2 receptor–mediated antinociceptive effects in rat models of acute inflammatory pain and chronic noninflammatory arthritic pain (136, 137). No undesirable effects in motility, catalepsy, or body temperature were observed for doses up to 10 mg/kg. PF-04457845 (1 mg/kg p.o.) reversed behavioral changes associated with postnatal lipopolysaccharide exposure (138), suggesting that social behavior impairments such as anxiety disorders or autism may benefit from FAAH inhibition. In addition, PF-04457845 did not provoke adverse effects in a rat model of working memory (139).

PF-04457845 was advanced to phase I trials to assess the pharmacology, tolerability, and bioavailability in humans [ClinicalTrials.gov identifier NCT00836082 (**https://www.clinicaltrials.gov/**)]. Pharmacokinetics demonstrated excellent ADME properties in humans (140). A single low dose (0.3 mg) inhibited >97% FAAH activity within 2 h. Chronic administration of PF-04457845 (0.5–8 mg) for 14 consecutive days induced prolonged elevation of AEA and other NAE levels for several days after the last dose (140). In a phase II trial, PF-04457845 was tested for efficacy in patients with inflammatory pain from osteoarthritis of the knee [NCT00981357, EudraCT (European Union Drug Regulating Authorities Clinical Trials Database) number 2009-014734-16 (**https://www.clinicaltrialsregister.eu/**)] (141). Patients were treated with 4 mg PF-04457845 for 14 days, which reduced FAAH activity by 96% and modulated endocannabinoid levels comparable to the phase I trial and without any adverse events (141). Despite prolonged elevation of NAE concentrations, PF-04457845 did not produce analgesic effects, while naproxen, a nonsteroidal anti-inflammatory agent commonly used by osteoarthritis patients, was effective.

The efficacy of PF-0457845 was also determined in cannabis use disorder in another phase II trial (NCT01618656) (142). Daily cannabis users were hospitalized for 5 days to generate abstinence and cannabis withdrawal, followed by a 3-week treatment of 4 mg PF-04457845 per day (142). Compared to placebo, PF-04457845 reduced symptoms of cannabis withdrawal and related mood symptoms. Furthermore, PF-04557845 produced fewer self-reported cannabis use events and lower urine concentrations of THC carboxylic acid, the major metabolite of THC. These outcomes underline the therapeutic potential of FAAH inhibition as an effective approach for the treatment of cannabis use disorder. This will be further explored in a subsequent clinical trial (NCT03386487).

PF-04457845 has also been evaluated as an anxiolytic drug (143). Administration of 4 mg PF-04457845 daily for 10 days to healthy subjects attenuated anxiogenic effects of stress, including negative affect and autonomic stress response, and prevented stress-induced decreases in AEA levels (143). Those results support the hypothesis that FAAH inhibition may be a potential therapeutic strategy for patients suffering from PTSD and other stress-related psychopathologies. Clinical phase II trials are currently assessing the anxiolytic efficacy of PF-04457845 (NCT01665573, 2016-005013-47) (**Table 1**).

SSR411298. SSR411298 is a reversible, selective, and potent FAAH inhibitor developed by Sanofi-Aventis (144) (**Table 1**). SSR411298 (3 mg/kg p.o.) increased AEA levels fivefold in mouse hippocampus. Testing the therapeutic activity of SSR411298 in rodent models of anxiety and depressive disorders revealed no effect on memory acquisition and consolidation in nonaversive tests, thereby showing no memory-impairing properties. Notably, in mice exposed to a stressor, SSR411298 normalized stress-induced deficiency in memory performance. The improvement of memory performance after stress underlines the potential of FAAH inhibition to treat traumatic fear memories. In models of anxiety, acute administration of SSR411298 showed varying effects depending on the stimulus used. No activity was found in tests addressing generalized anxiety and panic disorder, whereas SSR411298 produced effects on defensive aggression. These results

suggest that FAAH inhibition may be more useful for conditions of high stress following traumatic events. When SSR411298 was tested for antidepressant efficacy in the rat forced-swimming test and in a mouse chronic mild stress model, it exerted robust antidepressant-like activity and restored normal levels of anxiety. This preclinical data set supported the development of SSR411298 as a therapeutic to attenuate acute and chronic stress effects (144).

In 2008 and 2011, two phase II clinical trials were registered by Sanofi that were designed to evaluate the efficacy of SSR411298 in the treatment of major depressive disorder (MDD) in elderly patients (NCT00822744, 2008-001718-26) and as an adjunctive treatment for persistent cancer pain (NCT01439919, 2011-002557-56), respectively. No target engagement studies were reported. In the MDD trial, patients were given 10, 50, or 200 mg SSR411298 daily during an 8-week treatment period (145). SSR411298 did not show efficacy on depression or disability, anxiety, cognitive function, sleep, pain, and somatic symptoms related to depression, while the positive control group receiving 10 mg escitalopram showed significant antidepressive effects on the Hamilton Depression Rating Scale. The phase II trial for cancer pain was terminated due to the lack of eligible participants (146).

ASP8477. Astellas Pharma developed ASP8477 as a selective covalent FAAH inhibitor with high potency (147) (**Table 1**). ASP8477 (0.3–10 mg/kg p.o.) increased AEA levels in rat plasma and brain up to threefold. ASP8477 showed antinociceptive effects in capsaicin-induced secondary hyperalgesia, in two neuropathic pain models [L5/L6 spinal nerve ligation (SNL) and streptozotocin-induced diabetic model], and in an osteoarthritis model. No motor coordination deficits were observed at doses up to 30 mg/kg of ASP8477 (147).

A first-in-human trial assessed ASP8477 for safety, tolerability, and analgesic effects in comparison to duloxetine, an active control (NCT02220777) (148). ASP8477 was well tolerated across the dose range (20–100 mg), showed rapid absorption, and reached maximal concentrations within 2–4 h. AEA levels, as a marker for target modulation by ASP8477, were not reported. Capsaicin was topically applied to the skin as a human hyperalgesia model, which led to peripheral and spinal sensitization as measured by increased pain scores on the visual analog scale (VAS) and by laser-evoked potential (LEP) amplitudes. Multiple ascending doses of ASP8477 reduced LEP amplitudes but only significantly in subjects with positive capsaicin skin effects. Capsaicin-treated subjects reported significantly lower VAS pain scores after administration of ASP8477, but ASP8477 did not reach the maximal analgesic and antihyperalgesic effects observed with duloxetine (148).

In a clinical phase II trial, ASP8477 was tested for analgesic efficacy in patients with peripheral neuropathic pain (PNP) resulting from diabetic peripheral neuropathy or postherpetic neuralgia (NCT02065349, 2013-002521-27) (149). PNP patients received ASP8477 according to a titration period consisting of 10–20 mg twice per day (b.i.d.) for 3 days and a maintenance period of 20 or 30 mg b.i.d. for 21 days. Pharmacodynamic studies revealed that ASP8477 increased AEA levels by approximately sixfold. After this single-blind treatment period, responders to ASP8477, identified by a >30% decrease in daily pain intensity, were subjected to a subsequent double-blind period. Unfortunately, ASP8477 was ineffective in PNP patients at the end of the double-blind treatment period.

V158866. V158866 was developed by Vernalis as a reversible, potent FAAH inhibitor (150) (**Table 1**). In rats, maximal inhibition of carrageen-induced thermal hypersensitivity by V158866 (3 mg/kg p.o.) was comparable to the positive control: indomethacin (10 mg/kg p.o.). A first-in-human study was performed to evaluate the safety, tolerability, and pharmacology of V158866 after single and repeated ascending dosage for 7 days (NCT01634529) (151). V158866 showed an acceptable pharmacokinetic profile after single dosing up to 300 mg and repeated dosing (151).

Complete inhibition of FAAH activity occurred at ≥30 mg V158866 (single dose) and across the entire dose range for the repeated dosing study. Doses of 300–500 mg V158866 altered AEA plasma levels in accordance with its pharmacokinetic profile with peak levels maintained for 72 h. V158866 was evaluated in a clinical phase II trial for the treatment of central neuropathic pain due to spinal cord injury (NCT01748695). Patients receiving daily doses of 450 mg V158866 for 4 weeks did not report reduced pain intensity compared to placebo. The lack of efficacy led to the discontinuation of V158866.

PET: positron emission tomography

JNJ-42165279. In a 2011 patent application, JNJ-42165279 (Janssen Pharmaceutica) was reported to be a potent covalent inhibitor of FAAH with suitable pharmaceutical properties (152) (**Table 1**). JNJ-42165279 is slowly hydrolyzed by FAAH, thereby yielding partial return of enzyme activity over time (153). Preclinical characterization described JNJ-42165279 as a highly selective FAAH inhibitor with regard to other receptors, enzymes, transporters, and ion channels (153). Rats that were administered JNJ-42165279 (20 mg/kg p.o.) had a sufficient pharmacokinetic-pharmacodynamic profile, with maximal elevations of brain AEA fourfold over basal levels, which returned after 24 h (153). Analgesic efficacy was shown in the rat SNL model of neuropathic pain. Notably, mice that were orally administered the inhibitor (0.1 mg/kg) lacked inhibition of FAAH activity or modulation of NAE levels; thus, JNJ-42165279 was ineffective in mice (153, 154).

JNJ-42165279 has been extensively tested in clinical trials. Postnov et al. (155) reported results of two phase I trials (NCT01964651, NCT02169973) that evaluated target inhibition and occupancy by JNJ-42165279 using the FAAH positron emission tomography (PET) tracer MK-3168, developed by Merck. JNJ-42165279 (10–100 mg) produced at least 90% inhibition of peripheral FAAH in leukocytes after a single dose and up to 99% inhibition after multiple dosing for 10 days. NAE plasma levels were also elevated five-to-tenfold higher by both single and chronic dosing. Interestingly, in cerebrospinal fluid, chronic dosing with JNJ-42165279 produced 41–77-fold higher AEA levels compared to baseline. MK-3168 was rapidly metabolized in humans but showed high and uniform uptake over all gray matter regions after intravenous injection (155, 156). Pretreatment with JNJ-42165279 dose-dependently reduced MK-3168 binding in the brain (155). Target occupancy in the brain by JNJ-42165279, inferred from tracer plasma levels, reached 96–98% with a 10-mg dose and was maintained at >80% occupancy during the dosing interval. Use of MK-3168 enabled greater acceptable safety margins and lowered initial estimates of the minimum dose of JNJ-42165279 required for phase II studies. JNJ-42165279 has been tested for clinical efficacy in phase II trials for the treatment of major depressive disorder (NCT02498392, 2015-002007-29) and social anxiety disorder (NCT02432703) and is currently being tested in a trial for autism spectrum disorder (NCT03664232).

BIA 10-2474. A first-in-human study using the FAAH inhibitor BIA 10-2474 (Bial Pharmaceuticals) led to the death of one volunteer and hospitalization of four others who experienced mild to severe neurological symptoms (157) (**Table 1**). The volunteers received a total dose of 250–300 mg of the drug over 5–6 days. In view of the safety profile of the other FAAH inhibitors that were tested in clinical trials, it was suggested that off-target effects of BIA 10-2474 were responsible for the observed toxicity. Van Esbroeck et al. (158) used activity-based proteomics of human cerebral cortex and differentiated cortical neurons to identify the serine hydrolase interaction landscape. BIA 10-2474 inhibited several lipases (ABDH6, ABDH11, CES2, PLA2G15, and PNPLA6) that were not targeted by PF-04457845. BIA 10-2474, but not PF-04457845, produced substantial alterations in lipid networks. These findings were confirmed in another study (154). Although it cannot currently be concluded that inhibition of one or more of the identified off-target proteins was responsible for the clinical neurotoxicity, these findings suggest that promiscuous lipase

inhibitors have the potential to cause metabolic dysregulation in the CNS. The findings also stress the need for studies that determine on-target engagement and off-target activity of experimental drugs in a proteome-wide fashion using human cells and tissues to guide therapeutic development.

MAGL Inhibitors: ABX-1431

Therapeutic interest in MAGL inhibition is reflected by the number of patented MAGL inhibitors that have been developed by several pharmaceutical companies, including Janssen Pharmaceutica, Abide Therapeutics, Pfizer, Hoffman-LaRoche, and Takeda Pharmaceutical (159, 160). As of yet, only one MAGL inhibitor has successfully completed phase I clinical trials: ABX-1431.

ABX-1431, developed by Lundbeck (Abide Therapeutics), is a carbamate-based inhibitor that covalently binds to the active site Ser122 (161). The adduct is stable for at least 24 h. ABX-1431 displayed high potency for human MAGL and was highly selective, as determined by ABPP, showing little off-target activity for ABHD6 and PLA2G7 (161) (**Table 1**). ABX-1431 dose-dependently inhibited brain MAGL ($ED_{50} = 0.5–1.4$ mg/kg) with concomitant elevations in brain 2-AG levels after oral dosing in mice. Preclinical efficacy of ABX-1431 was assessed in the rat formalin pain model: a single dose (3 mg/kg) significantly reduced formalin-evoked paw licking duration (161).

ABX-1431 has thus far been tested in five clinical phase I trials for the treatment of hyperalgesia (NCT02929264), functional dyspepsia (NCT02875678), Tourette syndrome (NCT03058562), central pain (NCT03138421), and neuropathic pain (NCT03447756) (**Table 1**). Results are not available for the majority of these conditions, but positive data from the study in Tourette syndrome provided an incentive for continuing clinical evaluation of ABX-1431 in a phase II trial that is assessing efficacy in Tourette syndrome and chronic motor tic disorder (NCT03625453, 2018-000100-41) (162).

CONCLUSION

Medicinal cannabis has been used for various indications for centuries, but solid scientific evidence does not exist for the efficacy of cannabis in large-scale, double-blind, randomized, placebo-controlled clinical trials using objective clinical end points. Preparations containing isolated constituents of cannabis, such as Sativex (nabiximols), or synthetic compounds, e.g., Marinol (dronabinol) and Cesamet (nabilone), are approved for treating symptoms in MS patients, as antiemetics, and as appetite stimulants. Recently, Epidiolex (CBD) was approved to treat rare forms of pediatric epilepsy. Hopefully evidence-based investigations of other therapeutic applications of CBD and perhaps other phytocannabinoids will be conducted.

The discovery of the ECS has inspired many academic and industrial labs to develop alternative strategies for direct CB_1 receptor agonists. The inhibition of endocannabinoid hydrolysis by MAGL and FAAH is currently under investigation in clinical trials. Thus far, only one MAGL inhibitor has been tested in such trials; no (major) adverse events were described, and preliminary positive signs of efficacy were reported in patients with Tourette syndrome. While further clinical results are awaited, preclinical studies suggest that the dosing schedule of MAGL inhibitors should be carefully designed to avoid CB_1 receptor–mediated side effects. Acute and high levels of 2-AG may lead to psychotropic and cardiovascular effects, whereas long-term, chronic 2-AG elevation may lead to CB_1 receptor tolerance and downregulation, resulting in unwanted side effects associated with CB_1 receptor antagonism. It is hypothesized that reversible inhibitors may allow better control over MAGL inhibition compared to covalent, irreversible inhibitors (160). Interestingly, preclinical studies suggest that MAGL inhibitors may also be beneficial in cancer

and neuroinflammatory diseases without activating the CB_1 receptor but instead by preventing the formation of protumorigenic signals and proinflammatory prostaglandins, respectively.

Inhibitors of FAAH have taken center stage and have advanced to phase II clinical trials for multiple indications. FAAH inhibitors are considered to be safe experimental drugs that do not induce on-target toxicity. The clinical trial disaster with BIA 10-2474 highlights the need for preclinical selectivity testing (by using ABPP) as early as possible to detect off-target activities of (covalent) enzyme inhibitors. ABPP may also help guide dose selection in clinical trials. For most experimental drugs, solid evidence for FAAH inhibition and increased AEA levels was obtained in phase I and II clinical trials. The lack of efficacy in chronic pain patients thus cannot be attributed to a lack of target engagement and modulation and instead raises questions regarding the translation of results from preclinical pain models. Nevertheless, other patient groups may experience analgesic effects from (chronic) FAAH inhibition or, alternatively, by combination therapy with other analgesics such as opioids. Currently, the most promising therapeutic area for FAAH inhibitors appears to be the regulation of emotional disorders in relation to stress, anxiety, and fear extinction. The first preliminary evidence in humans from a randomized, controlled experimental trial using PF-04457845 has shown that FAAH inhibition improves recall of fear extinction memories and attenuates anxiogenic effects of stress. This warrants the testing of FAAH inhibitors in patients suffering from PTSD. Cannabis use disorder is another therapeutic application in which FAAH inhibitors have shown positive signs of efficacy. Improvement of withdrawal symptoms, such as lack of sleep, and reduced cannabis consumption were observed in a phase II trial (142).

Some aspects of FAAH and MAGL inhibition need to be addressed in (pre)clinical studies. For example, apart from the endocannabinoids, MAGL and FAAH hydrolyze other signaling lipids. What is the biological consequence of increasing the levels of these signaling lipids (163, 164)? Furthermore, the endocannabinoids can also serve as substrates for lipoxygenases and cyclooxygenase, which leads to the formation of bioactive metabolites with largely unknown functions (164). Are these oxygenated bioactive lipids produced in higher levels after (long-term) FAAH and MAGL inhibition, and if so, what is the biological effect?

In summary, while it is too early to conclude whether FAAH and MAGL inhibitors will emerge as successful drugs, results in the not too distant future are expected to reveal whether the hypothesis holds true that neither FAAH nor MAGL inactivation encompasses the complete spectrum of physiological effects induced by cannabis or a synthetic CB_1 receptor agonist. If so, one will be able to pharmacologically strengthen the endocannabinoid tone in the human brain in a spatiotemporally controlled manner (i.e., in only active local neuronal circuitries) that would not be possible with direct CB_1 receptor agonists. Such results will hopefully yield better alternatives than medical cannabis.

DISCLOSURE STATEMENT

M.v.d.S. has filed a patent application describing a chemical series as MAGL inhibitors, and he is a consultant for Hoffmann-LaRoche. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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