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The endocannabinoid system – current implications for drug development

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In this review, the state of the art for compounds affecting the endocannabinoid (eCB) system is described with a focus on the treatment of pain. Amongst directly acting CB receptor ligands, clinical experience with Δ^9-tetrahydracannabinol and medical cannabis in chronic non-cancer pain indicates that there are differences between the benefits perceived by patients and the at best modest effect seen in meta-analyses of randomized controlled trials. The reason for this difference is not known but may involve differences in the type of patients that are recruited, the study conditions that are chosen and the degree to which biases such as reporting bias are operative. Other directly acting CB receptor ligands such as biased agonists and allosteric receptor modulators have not yet reached the clinic. Amongst indirectly acting compounds targeting the enzymes responsible for the synthesis and catabolism of the eCBs anandamide and 2-arachidonoylglycerol, fatty acid amide hydrolase (FAAH) inhibitors have been investigated clinically but were *per se* not useful for the treatment of pain, although they may be useful for the treatment of post-traumatic stress disorder and cannabis use disorder. Dual-acting compounds targeting this enzyme and other targets such as cyclooxygenase-2 or transient potential vanilloid receptor 1 may be a way forward for the treatment of pain.

Keywords: Δ^9-tetrahydrocannabinol, anxiety, cannabinoid receptors, endocannabinoid, fatty acid amide hydrolase, pain.

Introduction

Cannabis sativa has been used for recreational and medicinal purposes for centuries [1]. Following the identification of the structure of Δ^9-tetrahydrocannabinol (THC), the main psychotropic ingredient in cannabis [2] and the identification and cloning of a cannabinoid (CB) receptor in the late 1980s–early 1990s [3,4], anandamide (arachidonoylethanolamide, AEA) was identified as an endogenous CB receptor ligand in 1992 [5] followed by 2-arachidonoylglycerol (2-AG) in 1995 [6,7] (for a review of the discovery of the structure of plant-derived (phyto-) and endogenous cannabinoids, see [8]). Since then, a massive scientific effort has delineated the 'endocannabinoid' (eCB) system, and how it can be modulated pharmacologically. In the present review, the current state of the art with respect to drug development in the eCB system is discussed, primarily with respect to the treatment of pain.

The eCB system

In this review, the eCB system is defined as the CB receptors, the main eCB ligands AEA and 2-AG, and their synthetic and degradative enzymes. This can be considered as the minimalist approach, since other endogenous CB ligands have been described [8], as have other targets for AEA and 2-AG, such as the transient potential receptor vanilloid 1 (TRPV1) [9] (for a review of the extended eCB system, see [10]), but it is of necessity in order to keep this review to a manageable size. Using the minimalist definition, pharmacological manipulation can be considered in terms of directly acting compounds (*i.e.* those interacting directly with the CB receptors as agonists, neutral antagonists, inverse agonists, biased agonists, or allosteric modulators) and of indirectly acting compounds (*i.e.* those affecting the concentration of eCBs available to interact with the receptor). These will be considered, in turn, together with a description of the targets themselves.

CB receptors

The name 'CB receptors' is something of a misnomer, since it implies that it is a receptor for cannabinoids. Whilst this of course is true for eCBs and for synthetic cannabinoids designed to target the receptor, most of the 110 or so phytocannabinoids [11] do not in fact interact with CB receptors. The International Union of Pharmacology recommends that novel receptors are named after 'the endogenous agonist, or the appropriate collective term when a family of related substances may interact with the receptor' [12]. CB receptors are admittedly not novel, but naming them after the endogenous agonist with the highest efficacy would suggest the use of 2-AG_1 and 2-AG_2 for CB_1 and CB_2 receptors, respectively. Such a change in nomenclature would resolve the confusion, but is unlikely to be particularly popular.[1]

CB_1 receptors, the receptor subype mediating the psychotropic effects of THC, are found in high abundance (at concentrations similar to those of striatal dopamine receptors) in the brain [13]. The distribution is, however, heterogeneous, with high expression being found in regions such as the substantia nigra, moderate expression in the hippocampus and low expression in the thalamus and pons [13]. The distribution of receptors reflects the myriad pharmacological effects of THC on perception, cognition, anxiety, gait, *et cetera* [14,15], whilst the low expression in the medullary nuclei [13] means that respiration is largely unaffected. CB_1 receptors are found on presynaptic nerve terminals, where they regulate neurotransmitter release, and a key role of 2-AG in the brain is to act as a retrograde transmitter, whereby postsynaptic 2-AG release results in activation of presynaptic CB_1 receptors which in turn inhibit the release of the neurotransmitter from the presynaptic nerve terminal [16] It would be wrong, however, to consider CB_1 receptors as exclusively neuronal in the brain, since functional CB_1 receptors are also expressed on astrocytes [17] It would also be incorrect to consider CB_1 receptors as being restricted to the brain and spinal cord – they have a wide distribution in the periphery with important functional properties in, for example, the autonomic nervous system, the gastrointestinal tract, adipose tissue, and bone [18-20] CB_2 receptors, first cloned in 1993 [21], are primarily found in immune cells (including microglia) but are also found in sensory, enteric and some central neurons [14,22].

CB receptors are G protein coupled receptors coupling primarily to G_i/G_o and producing inhibitory effects on adenylyl cyclase and calcium channels as well as activating potassium channels and the mitogen activated kinase pathway [14]. However, the signalling produced by CB receptor activation is nuanced, given the presence of functionally active intracellular receptors [23,24], receptor heterodimerization [25], regulation by receptor-interacting proteins [26], coupling to different signalling pathways at different receptor expression levels [27] and negative-feedback mechanisms [28]. This has been extensively investigated in tumour cells, where both mitogenic and apoptotic effects mediated by CB receptors have been reported in the literature (e.g. [29,30]; for schematics showing the complex effects of cannabinoids upon intracellular signalling and the results thereof in cancer cells, see Fig. 1 of [31] and [32]).

A final note in this section concerns possible additional CB receptors, such as the orphan receptor GPR55, which, when transfected into human embryonic kidney cells, was originally reported to bind and respond (increased GTPγS binding) to THC, AEA, 2-AG and some synthetic cannabinoid receptor ligands [33]. However, these data are controversial [34] and the International Union of Pharmacology, in their review in 2010, argued that the current data were insufficient to warrant the expansion of CB receptors to include additional receptors [35], and GPR55 remains an orphan receptor [36].

CB receptor ligands

Table 1 shows a selection of CB receptor ligands based upon their source and pharmacological effects. The discovered ligands run the entire gamut from pure agonists to inverse agonists and have been invaluable in characterizing the roles played by CB_1 and CB_2 receptors in the body. For readers unused to the terms 'inverse agonists' and 'biased agonists', see Fig. 1 for a mechanistic explanation.

[1]At the 20th annual symposium of the International Cannabinoid Research Society held in Lund, Sweden in 2010, a lighthearted debate with the title "to CB or not to CB" discussed this issue. The assembled delegates voted that in their view the present nomenclature should be kept, despite the best efforts of this author and others on the "not to CB" team to persuade them otherwise.

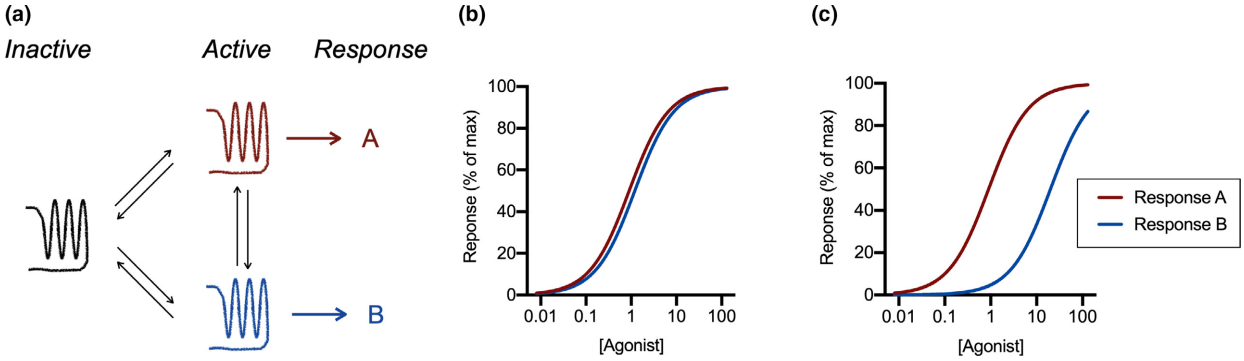

Fig. 1 *Different conformations of G-protein-coupled receptors and their responses. In this simple example (Panel a), the receptor is in three conformations: an inactive and two active conformations that couple to different transduction pathways and responses. These conformations are in equilibrium with each other, and in 'rest' conditions, most of the receptor is usually in the inactive form.* Agonists *have high affinities for the active forms and shift the equilibrium to the right (i.e. the active forms), whereas* inverse agonists *have high affinities for the inactive form and shift the equilibrium to the left.* Neutral antagonists *are high-affinity compounds that bind to all forms with equal affinities and thus do not move the equilibrium, but prevent agonists from doing so.* Biased agonists *bind with different affinities to the active forms and thus favour one of the active forms [186]. This means that in theory the concentration-response curves for response A and B are similar for agonists ('balanced agonists', Panel b), but different for biased agonists (Panel c). In practice, the curves for balanced agonists are not necessarily the same due to factors such as coupling efficiencies, expression of target response proteins etc, and so the degree of bias is often described relative to a standard compound (see [187] for examples with the CB_1 receptor, where Response A is recruitment of an engineered mini-$G\alpha_i$ protein and Response B is recruitment of β-arrestin2).*

The prototypical CB receptor ligand is of course THC, which acts as a partial agonist at CB_1 and CB_2 receptors [37]. This differs from the synthetic compounds such as 5F CUMYL-PICA and MDMB-FUBINACA and MDMB-CHIMICA which have been rationally designed as recreational drugs [38] and which generally have greater efficacy than THC [38–40]. This difference in efficacy, together with potential off-target effects of the compounds *per se* and/or their metabolites and impurities in the preparations, accounts for the more severe adverse effects of such compounds, including 'zombie-like' behaviours, hallucinations, neurological disturbances and possibly even death [41–43].

Clinical use of THC, cannabidiol (CBD) and cannabis-based medicines

Synthetic THC (dronabinol) and nabilone, a close analogue of THC, have long been used for the treatment of anorexia in AIDS patients and for chemotherapy-induced nausea and vomiting, and more recently, nabiximols (an oromucosal spray with plant-derived THC and CBD) has been approved for the adjunctive treatment of spasticity in multiple sclerosis and in Canada for the treatment of pain associated with multiple sclerosis and cancer. CBD has recently been approved as an orphan drug in Europe and the USA as an add-on treatment of Dravet and Lennox-Gastaut syndromes although its efficacy is more likely due to effects upon ion channels than upon CB receptors [44-46]. Pharmacokinetic interactions with the first-line drug clobazam secondary to inhibition by CBD of CYP3A4 and CYP2D6 have been described (see [46]), but the clinical significance of this with respect to the beneficial effects of CBD in these rare syndromes is as yet unclear.

There is an increasing acceptance in different countries, driven more by societal rather than by rigorous evidence-based scientific considerations, to allow the compassionate use of medicinal cannabis. The degree of such acceptance varies considerably from country to country (see [47] for a discussion with respect to the status in Europe of cannabis-based medicines for the treatment of chronic pain as of 2017). An important aspect is the difference in benefit as perceived by specialists and by the patients themselves. Thus, with respect to the use of cannabinoids and cannabis for non-cancer pain, a recent meta-analysis [48] of 47 randomized controlled trials reported a number needed to treat (NNT) for a 30% reduction pain of

Table 1. *CB receptor ligands*

Mechanism	Endogenous compounds	Phyto-cannabinoids	Synthetic ligands
Partial non-selective agonist	AEA [188–190]	THC [37]	
Full non-selective agonists	2-AG [189,190]		CP55,940 [188] 5F CUMYL-PICA [39]
Selective CB_1 receptor agonists			ACEA [191] O-1812 [192]
Selective CB_2 receptor agonists			JWH133 [193] A-796260 [61]
Biased CB receptor agonists			EG-018 [187] PNR-4-20 [70] LY2828360 [72]
Non-selective neutral antagonist		THCV[a] [194]	
CB_1 receptor-selective neutral antagonist			AM4113 [195]
Selective CB_1 receptor inverse agonists	Hemopressin [196]		Rimonabant (SR171416A) [197] AM251 [198]
Selective CB_2 receptor inverse agonists			SR144528 [199] AM630 [200]
CB_1 receptor allosteric modulators	Pregnanolone [28] Lipoxin A4 [201]	CBD[b] [202]	Org27569 [203] PSNCBAM-1 [204] GAT100 [205]

The references refer to the characterization of efficacies of the compounds rather than to their first discovery. Thus for example, rimonabant was initially described as a CB_1 receptor-selective antagonist [206], before being recategorized later as an inverse agonist. Note also that *in vitro* efficacies are not always mirrored *in vivo* (see [45]).
[a]Δ^9-tetrahydrocannabivarin; efficacy refers to CB_1 receptors.
[b]Cannabidiol.

24 (95% confidence interval 15–61); for a 50% reduction in pain, the active treatment was not significantly better than placebo. Put another way, the benefit corresponded to ~3 mm greater than placebo on a 100 mm visual analogue scale [48]. The number needed to treat to harm (with respect to all-cause adverse effects) was 6 (95% confidence interval 5–8). The authors concluded that 'it appears unlikely that cannabinoids are highly effective medicines for CNCP' (chronic non-cancer pain) [48]. Contrast this with a survey of 1748 Australian participants using cannabis preparations for pain, mental health, sleep problems and neurological conditions, where a very large majority of participants reported very much or much improved symptoms as a result of their cannabis use [49]. Their reported adverse effect profile (approximately $^3/_4$ of the participants reported increased appetite; $^2/_3$ drowsiness; $^2/_5$ ocular irritation; $^3/_8$ lack of energy; $^1/_3$ memory impairment; $^1/_6$ palpitations, paranoia; $^1/_8$ confusion, decreased appetite; $^1/_{10}$ dizziness; $^1/_{10}$ anxiety being the most common [49]) was the expected profile for THC-containing preparations [14,15]. The study was undertaken prior to Australian legislation allowed prescription of cannabis-based medicines, but a follow-up study taken after the legislation produced similar findings, albeit with the interesting observation that only 2.4% of the participants had used legally prescribed medical cannabis [50]. There were several reasons for this, but almost half of the respondents stated that they did not know a medical practitioner willing to subscribe medicinal cannabis, and $^1/_8$ did not

want the healthcare providers to know about their use of medicinal cannabis. This is problematic, since it raises the spectre of prescribed drug-medicinal cannabis interactions, not least secondary to the inhibitory effects of CBD on the CYP oxidase enzymes, that could be detrimental to the well-being of the patient.

To readers who are not clinicians (including this author, who is a basal pharmacologist by trade), the large difference between the findings of [48] and [49] may be puzzling. Randomized clinical trials, when conducted well, provide good evidence for efficacy without problems of bias, but the patients that are recruited and the study conditions that are implemented may not mirror the real world. On the other hand, studies like [49] show use of medicines that unselected patients have chosen and, in this case, dosed individually to suit themselves, but bias such as selection and reporting bias is a real issue (see Table 2 as a theoretical illustration, with emphasis on the word 'theoretical', of reporting bias).

Whatever the explanation(s) for the difference between [48] and [49] (and other studies), they highlight a gap between recommendations of health specialists (for example, the Faculty of Pain Medicine of the Australian and New Zealand College of Anaesthetists who state 'At the present time, the scientific evidence for the efficacy of cannabinoids in the management of people with chronic non-cancer pain is insufficient to justify endorsement of their clinical use' [51]) and changes in the legal status of medicinal cannabis in many countries [47], with general practitioners caught in the middle [52,53]. Two quotes are worth citing to illustrate this dilemma: 'Unless we learn from the history of opioids and their use, we run the risk of replicating a non-evidence based approach to pain

Table 2. *Simulated data set to illustrate reporting bias*

Range (units)	(≥+2)	(+1.2 to + 1.99)	(+0.4 to + 1.199	(−0.4 to + 0.39)	(−1.2 to −0.41)	(−2 to −1.21)	(<−2)
'Perceived effect'	+++	++	+	±0	-	--	---
Number [%] of individuals:							
Treatment paradigm (score 0 ± 1)	104 [2%]	450 [9%]	1153 [23%]	1585 [32%]	1122 [22%]	463 [9%]	123 [2%]
+ placebo effect (score 1 ± 1)	762 [15%]	1394 [28%]	1528 [31%]	936 [19%]	312 [6%]	58 [1%]	10 [0.2%]
% of participants responding to questionnaire ('repliers')	90%	60%	10%	5%	5%	10%	30%
+ placebo effect x repliers	686 [39%]	836 [48%]	153 [9%]	47 [3%]	16 [0.9%]	6 [0.3%]	3 [0.2%]

The simulation models a situation where a questionnaire is posted targeting individuals who have accessed a treatment paradigm without involvement of the health profession. A series of 5000 randomly generated data points following a normal distribution of 0 ± 1 (standard deviation) was used to simulate a perceived change following the treatment paradigm for 5000 individuals, assuming that the paradigm is without any benefit. The number [%] of data points within the ranges shown in the Table are determined, with each range being characterized as a given level of 'perceived benefit'. Next, an average placebo effect of + 1 unit is included (by random generation of 5000 data points following a normal distribution of +1 ±1), which increases the total % of cases in the +++ and ++ groups from 11% (treatment paradigm) to 43% (treatment paradigm + placebo effect). Finally, a reporting bias has been added, assuming that the individuals with extreme outcomes in the generated data are more likely to fill out the questionnaire ('repliers') to tell people about the beneficial effects, or to warn people about the negative outcome, than those where outcomes were marginal at best. The numbers shown in the '+ placebo effect x repliers' are the + placebo effect data multiplied by the % repliers, and is the result that study designers would obtain. The % reply rates chosen are not unreasonable if the treatment paradigm in question was illegal at the time of the study, so participants may have been reticent to respond to the survey unless they felt that the outcomes were extreme and thereby worth reporting. With these reply rates, the % of cases in the +++ and ++ groups is 87% of the total number of repliers. It is important to stress that this simulation is not designed to dismiss studies of this type as merely reflecting a placebo effect + a reporting bias, but rather to highlight the importance of consideration of bias in their interpretation.

management (with cannabis, my note), which will ultimately let down patients in need' [54] and 'important that health providers understand that their patients' experiences of medical cannabis may not accord with reported clinical findings' [49].

Two other issues should be mentioned: whether or not THC enhances the analgesic effects of opioids and whether or not the changes in legislation have impacted the opioid epidemic in the USA. With respect to the former, population and observational studies have given conflicting results [55,56], and in their review, Babalonis and Walsh [57] concluded that 'the extant controlled clinical data do not support the role of cannabinoids for opioid replacement or opioid-sparing effects when treating opioid use disorder or chronic pain'. With respect to the latter, data suggesting this to be the case in USA states that adopted medical cannabis early was not found in states that changed their medical cannabis laws at a later date, possibly because of the degree of regulation of dispensaries was different in the later states [58]. The type of opioid in question was also an important parameter in determining the impact of cannabis on opioid-based overdoses and deaths [58].

Peripherally restricted CB receptor agonists and CB_2 receptor-selective agonists

The unwanted central effect profile of THC places a ceiling on dosage. In order to avoid this issue, a peripherally restricted CB_1 and CB_2 receptor agonist, AZD1940, was developed and investigated clinically in acute pain (lower third molar surgical removal, capsaicin-induced pain and hyperalgesia) [59,60]. In both cases, the effects of AZD1940 were not better than placebo, a perhaps unsurprising result given the doubtful efficacy of THC itself in acute pain [57].

CB_2 receptor-selective agonists do not produce the central effects of THC and are in theory a potentially attractive approach to therapies whereby engagement of this target can lead to beneficial outcome. At the outset, this approach looked very promising, with several different CB_2 receptor-selective agonists producing beneficial effects in animal models of chronic inflammatory, neuropathic, postoperative and osteoarthritic pain [61–64]. However, the trail ended at the clinic. To my knowledge, the only published clinical studies for a CB_2 receptor-selective agonist or biased agonist are those of Ostenfeld *et al.* [65] who reported that GW842166 was not efficacious in acute pain (third molar extraction) and Pereira *et al.* [66] who in an abstract reported a lack of efficacy of LY2828360 in osteoarthritic knee pain. The 'loss in translation' between preclinical promise and clinical reality is not restricted to CB_2 receptor agonists but is a general problem [67]. One of several factors in play concerns the predictive validity of the preclinical animal models, which usually measure evoked hypersensitivity, which is only one (and not the most important) aspect of the pain seen in human neuropathic pain [68] (for a discussion of the preclinical and clinical disparity with respect to CB_2 receptor agonists, see [69]).

Biased agonists as a way forward?

The notion that biased agonists can produce different degrees of activation along different pathways is attractive for drug development, particularly if such an approach can discriminate beneficial from adverse effects. CB receptor biased agonists have been investigated with respect to tolerance upon chronic use. Thus Ford *et al.* [70] described a non-selective CB receptor agonist, PNR-4-20, that stimulated G-protein mediated signalling but was less efficacious for β-arrestin 2 recruitment in Chinese hamster ovary cells transfected with human CB_1 receptors. β-arrestin 2 recruitment correlates with agonist-induced internalization of CB_1 receptors [71], and PNR-4-20 treatment of the cells caused and less down-regulation and desensitization of CB_1 receptors than the 'balanced' agonist CP-55940 [70]. Another compound, LY2828360, produced biased signalling and a lower degree of CB_2 receptor internalisation than CP55,940 [72]. *In vivo*, PNR-4-20 produced less tolerance to its hypothermic effect than seen with THC and the non-selective agonist JWH-018, and also produced less inverse agonist-induced withdrawal symptoms than JWH-018 following repeated exposure [70] Although early days, these data suggest that a biased partial CB_1 receptor agonist might be useful as a THC-mimic but with more moderate issues of tolerance and withdrawal effects than THC itself.

CB_1 receptor inverse agonists and allosteric modulators

The pharmacology and clinical outcome of CB_1 receptor inverse agonists and potential follow-up compounds has been reviewed recently [19] and so will only be dealt with briefly here. The well-known effects of THC upon appetite (the 'munchies') raises

the possibility that blockade of eCB signalling could provide a useful way to produce weight reduction. Over the years, it has been established that the eCB system effects food intake and metabolism not only due to central mechanisms mediated by CB_1 receptors, but also by peripheral mechanisms, not least due to the CB_1 receptor expression on adipocytes [19]. A series of clinical trials led to the approval in Europe in 2006 of rimonabant for the treatment of obesity. Other CB_1 receptor-selective inverse agonists (taranabant, otenabant, ibipinabant and surinabant) were also undergoing clinical trials for treatment of obesity and as an aid to smoking cessation. However, the field imploded when rimonabant was withdrawn from the market due to an unacceptable risk of psychiatric side-effects, in particular anxiety and depressive symptoms [73]. It is possible that peripherally restricted CB_1 receptor antagonists / inverse agonists may be a way around this issue, and such compounds have been described in the literature [19]. An alternative approach is the use of CB_1 receptor allosteric negative modulators, which produce a less draconian modulation of CB_1 receptor signalling than inverse agonists, and thereby may produce a more acceptable wanted : adverse effect profile [74], the operative word here being 'may'.

Endocannabinoid synthesis and degradation: targets for indirectly acting compounds

AEA belongs to the family of endogenous *N*-acylethanolamines (NAE) and the canonical pathway for NAE synthesis was established by Schmid and colleagues well before AEA was discovered [75–77]. In this pathway, *N*-acylphosphatidylethanolamines (NAPEs) are formed by the transacylation of membrane phosphatidylethanolamine containing phospholipids. NAPEs are then hydrolysed by NAPE-hydrolysing phospholipase D (NAPE-PLD) to form the NAEs (see Fig. 2). Three comments should be made:

- Genetic deletion or inhibition of NAPE-PLD reduces NAE levels in the brain, and its inhibition influences emotional behaviour in mice [78,79]. However, there are alternative synthetic pathways (reviews, see [80,81]).

- AEA synthesis is 'on demand' and is controlled by the environment. Thus, for example, treatment of cortical neuronal cultures with the combination of glutamate and the acetylcholine receptor agonist carbachol increases AEA formation in a manner blocked by buffering of intracellular calcium [82]. NAPE-PLD itself can be regulated by bile acids [83] and inflammatory stimuli [84].

- As implied by the fact that the canonical pathway was established prior to the discovery of AEA, the output of the pathway is not AEA alone but a family of NAEs, of which the most abundant are palmitoylethanolamide (PEA), oleoylethanolamide (OEA) and stearoylethanolamide (SEA). Indeed, in most tissues, levels of PEA, OEA and SEA are much higher than the levels of AEA, a notable exception being the mouse uterus, where AEA is predominant and where its levels are inversely associated with uterine receptivity [85]. These compounds do not interact directly with CB receptors. However, they are biologically active, the most studied being PEA which produces anti-inflammatory effects mediated by activation of peroxisome proliferator-activated receptor α and other targets, and which has been reported to have beneficial effects upon pain in humans (reviews, see [86,87]).

The synthesis of 2-AG (shown schematically in Fig. 2) has similarities to AEA synthesis in that there is a canonical pathway mediated by diacylglycerol lipases (DAGLs) as well as alternative pathways [80,81]; that its synthesis and release is on demand although release from preformed pools has been postulated [88]; that synthesis of 2-AG is accompanied by synthesis of close homologues such as 2-oleoylglycerol and 2-palmitoylglycerol that can modulate the activity of 2-AG [89,90]; and that the expression of DAGL, at least at the level of mRNA, can be regulated by inflammatory mediators [91]. In the brain, DAGL inhibition affects retrograde signalling and neuroinflammatory responses [92,93].

The mechanism(s) responsible for the release and uptake of eCBs have been a matter of contention for many years. The two current trains of thought are that there is a bidirectional transport across the plasma membrane that is either mediated by an as yet unknown protein or alternatively that the current evidence is consistent with simple diffusion across the plasma membrane [94,95]. What is clear is that following uptake, AEA is transported within the cell by carrier proteins such as fatty acid binding protein 5, and that an inhibitor of this protein, when given intracerebroventricularly, increases brain AEA, OEA and PEA but not 2-AG levels [96]. WOBE437, a potent inhibitor of AEA

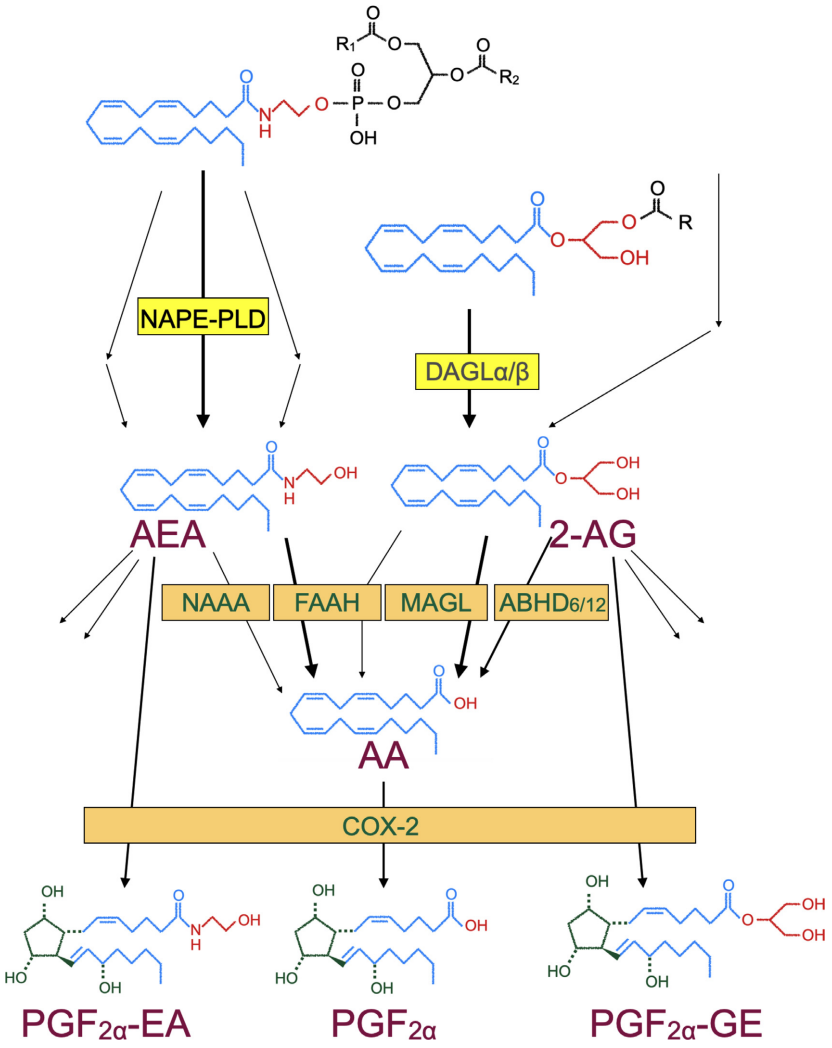

Fig. 2 *AEA and 2-AG turnover starting from the appropriate NAPE and diacylglycerol (DAG), respectively. The thick arrows show the canonical pathway, with alternate pathways (reviewed in [80,81]) for the synthesis and degradation being shown with the thin arrows. Abbreviations (when not given in the text): AA, arachidonic acid; EA, ethanolamide; GE, glyceryl ester. Note that the PG-EA, PG and PG-GE species shown is $F_{2\alpha}$, but the corresponding D_2 and E_2 species are also formed. Note also that the PG-GEs rapidly isomerize to form the corresponding PG-1-GEs, and this form dominates in PG-GEs preparations that are commercially available.*

uptake has been described and shown to be active in a mouse model of monoarthritis [97].

Much more is known about the hydrolytic enzymes for AEA and 2-AG. Fatty acid amide hydrolase (FAAH), was discovered and characterized originally with PEA and OEA as substrates [98,99]. It has a wide substrate specificity and can hydrolyse *N*-acylamides and *N*-acyltaurines as well as NAEs [100,101]. Examples of FAAH inhibitors (of which there are many [102]) are shown in Table 3.

A second FAAH, termed FAAH-2, has been identified [103][2]. The enzyme, which is found in humans

[2]FAAH-2 is somewhat the poor cousin to FAAH in terms of research interest: a PubMed search conducted in October 2020 with the search term "FAAH-2" returned 7 results, as compared with 1706 results for FAAH.

Table 3. *Examples of FAAH, MAGL (monoacylglycerol lipase) and dual-action inhibitors*

Enzyme targeted	Reversible inhibitors	Irreversible inhibitors
FAAH-selective	OL-135 [126]	URB597 [133]
	AZ513 [207]	PF-3845 [127]
	SSR411298 [208]	PF-04457845 [128]
		JNJ-1661010 [129]
		JNJ-42165279 [130]
MAGL-selective	Compound 21 of [209][a]	JZL184 [212]
	Compound 20b of [210][b]	KML29 [213]
	Compound 26 of [211][c]	ABX-1431 [176]
FAAH/MAGL	Compound 8 of [214][d]	JZL195 [110]
FAAH/TRPV1	*N*-arachidonoylserotonin [164][e]	OMDM-198 (?) [165][f]
FAAH/COX	Ibu-AM5 [150]	ARN2508 [152]
	Flu-AM1 [151]	
FAAH-sEH[g]		Compound 11 of [169][h]

For the FAAH irreversible compounds, the irreversibility is generally determined by use of dialysis or substrate dilution experiments and the demonstration of time-dependent inhibition. However, use of long dialysis times suggest that compounds like JNJ-1661010 may be a slowly reversible compound despite covalent interaction with FAAH [215]. ASP3652 has been described as such a compound [216], although to my knowledge the data supporting this claim has not been published.

[a]Benzo [*d*][1,3] dioxol-5-ylmethyl 6-([1,1'-biphenyl]-4-yl)hexanoate.

[b](*Z*)-4-{[4,40"-dimethoxy-(1,1':4',1"-terphenyl)-2'-yl]methylene}-2-methyloxazol-5(4*H*)-one.

[c](4-Benzylpiperidin-1-yl)(5-(4-hydroxyphenyl)-1-(3-methylbenzyl)-1*H*-pyrazol-3-yl)methanone.

[d](±)-oxiran-2-ylmethyl 6-(1,1′-Biphenyl-4-yl)hexanoate.

[e]Reference is for the first report of its actions towards FAAH.

[f]OMDM-198 is compound 10 in this reference. The mechanism of action was not determined, hence the question mark by the name, but I have classified it as irreversible on the basis of it being a carbamate compound.

[g]Soluble epoxide hydrolase.

[h]*N*-(4-(trifluoromethoy)phenyl)-4-(3-((5-(trifluoromethyl)pyridin-2yl)oxy)benzyl)piperidine-1-carboxamide.

but not in mice or rats, hydrolyses oleamide as effectively as FAAH. However, this is not the case for AEA. Defining the rate of oleamide as unity for both enzymes, the relative rates of hydrolysis of AEA are 1.75 and 0.054, respectively [103]. A third hydrolytic enzyme, *N*-acylethanolamine-hydrolysing acid amidase (NAAA) was described at the turn of the century. This enzyme, which unlike FAAH has a pH optimum ~ 5 (as opposed to ~ 9 for FAAH), is found at high concentrations in macrophages, and hydrolyses PEA more avidly than AEA [104]. The relative activities of FAAH, FAAH-2 and NAAA are shown in Fig. 3a and examples of the relative selectivity of some FAAH inhibitors for this enzyme vs. FAAH-2 are shown in Fig. 3b.

The ability of the different enzymes to hydrolyse AEA means at least in theory that their relative contribution in a given tissue will be dependent upon their relative expression. This has not been explored in any great detail, but in T84 human colon cells, expression of FAAH and NAAA is very similar at the mRNA level, but inhibition of FAAH by URB597 produces a robust increase in AEA and other NAE levels, whereas the NAAA inhibitor pentadecylamine produces a much smaller, albeit significant increase [105]. *In vivo*, treatment with either URB597 or PF-3845 increases AEA but not PEA levels in the colon of mice with trinitrobenzene sulfonic acid-induced colitis, whereas the reverse is seen following treatment with the NAAA inhibitor AM9053 [106].

FAAH can also hydrolyse 2-AG [107] although in the brain, the primary hydrolytic enzyme is monoacylglycerol lipase [108]. The development of FAAH and MAGL-selective inhibitors (reviewed in [102], see Table 3) has provided an invaluable tool in

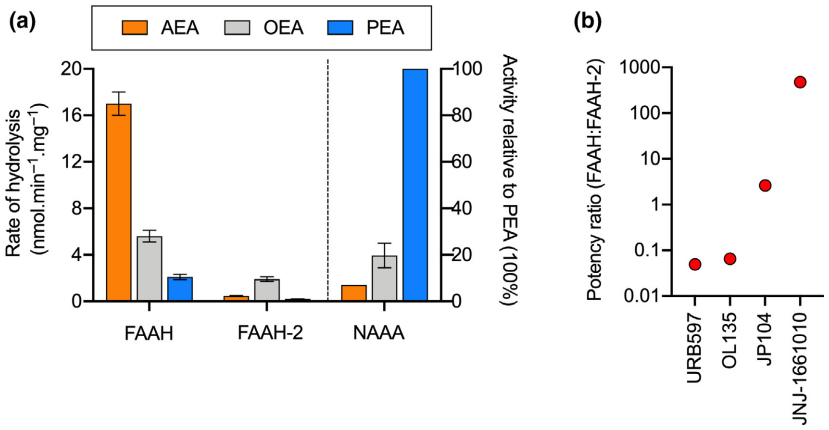

Fig. 3 *Panel (a) relative rates of hydrolysis of the NAEs PEA, OEA and AEA by FAAH, FAAH-2 and NAAA. The data for FAAH and FAAH-2 were taken from Table 2 of Wei* et al. *[103] who used COS-7 cells transfected with human FAAH-1-pcDNA3 or FAAH-2-pFLAG.CMV6 constructs and an assay pH of 9 (the pH optimum for FAAH). The data for NAAA (right axis) were estimated from Fig. 7 of Ueda* et al. *[104] who used NAAA purified from rat lung enzyme and an assay pH of 5 (in the presence of Triton X-100) and expressed the activity with AEA and OEA relative to that with PEA as substrate. The error bars (when not too small to be visible in the original study [AEA for NAAA] or when the activity was set to 100% [PEA for NAAA]) represent SD. Panel (b) shows the potency ratio of four inhibitors towards FAAH and FAAH-2, calculated from [103] and [129]. The higher the number, the greater the selectivity towards FAAH.*

demonstrating that AEA and 2-AG are not simply alternate eCBs within a given system, but in fact play separate physiological roles. Thus, for example, in rodents trained to discriminate THC from vehicle, URB597 does not substitute for THC [109]. In mice, the MAGL-selective inhibitor JZL184 produces a partial substitution for THC whilst KML29 showed no substitution [110,111]. However, administration of either a non-selective FAAH/MAGL inhibitor (JZL195), the combination of URB597 and JZL184, or the administration of JZL184 to $FAAH^{-/-}$ mice substituted for THC in this test [109,110].

Two other hydrolytic enzymes, the α/β-hydrolase domain 6 and 12 (ABHD6 and 12) are also shown in Fig. 2. In brain homogenates, they are minor contributors to 2-AG hydrolysis [108]. However, in mouse brain neurons in primary culture, ABHD6 contributes significantly to 2-AG hydrolysis [112]; even more so in the mouse BV2 microglial cell line where MAGL is not expressed [112]; and in the human SH-SY5Y neuroblastoma cell line, the mRNA levels of *ABHD6* and *ABHD12* are much greater than those of *MGLL*, coding for MAGL [113]. The ABHD6 inhibitor WWL70 produces biological effects *in vivo*, but interpretation of these effects is hampered by an off-target effect of the compound upon the biosynthesis of prostaglandin E_2 [114]. However, other inhibitors have been developed [115] and will hopefully give more information as to the importance of this 2-AG hydrolytic pathway *in vivo*. Mutations in ABHD12 cause PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract), a neurodegenerative disease [116]. ABHD12 can hydrolyse other lipids in addition to 2-AG, not least lyso-phosphatidylserine, and a recent paper utilizing mice lacking ABHD12 and the lysophospholipid acyltransferase LPCAT3 has suggested that dysfunction in the regulation of lyso-phosphatidylserines underlies PHARC [117].

The above discussion has considered the hydrolytic enzymes as ways to remove AEA and 2-AG, but MAGL also has an anabolic function. Thus, in several cancer cell lines, MAGL acts to catalyse the production of long-chain fatty acids from the corresponding monoacylglycerols, and this provides an energy source for the cells that aids their proliferation *in vivo* in xenograft models [118,119]. In the brain, MAGL-catalysed hydrolysis of 2-AG provides a key source of arachidonic acid needed for prostaglandin production in neuro-inflammatory disorders [120]. AEA and 2-AG are also substrates for cyclooxygenase-2 (COX-2),

Table 4. *Results of Phase 2 clinical trials for FAAH inhibitors in pain patients: efficacy data*[a]

FAAH inhibitor (comparator)	ClinicalTrials.gov protocol; trial design	Disorder; Dose regime	Registered primary aim	Outcome of primary aim	Comments	Ref.
PF-04457845 (naproxen) (placebo) Sponsor: Pfizer	NCT00981357; randomized double-blind double-dummy crossover: drug followed by placebo and *vice versa*	Osteoarthritis of the knee; PF: 4 mg once daily Nap: 500 mg b.i.d.	Efficacy *vs.* placebo in the WOMAC[b] pain sub-score; safety and tolerability of PF-04457845 in the patients	PF-04457845 ($N = 35$) showed no reduction in the WOMAC pain sub-score compared to placebo after 2 weeks of treatment. Naproxen was effective at a decision criterion '90% sure the compound is better than placebo' ($N = 36$)	The dose regime used gave a large (>95%) reduction in leukocyte FAAH activity and increased plasma NAE levels (12-fold for AEA, 3.3-fold for PEA and 8.9-fold for OEA). The per cent of patients who used rescue medication was the same in the placebo and PF-04457845 arms of the trial (59%)	[149]
ASP3652 (placebo) Sponsor: Astellas Pharma Inc.	NCT01391338; randomized double-blind adaptive parallel assignment	Men with chronic abacterial prostatitis or chronic pelvic pain syndrome; 25, 75, 150, 300 mg b.i.d. or 300 mg once daily	Change from baseline in the NIH-CPSI[c] total score after 12 weeks of treatment	For all five dose regimes ($N = 26$–52), no significant difference in the change in baseline to that seen with placebo ($N = 53$) was found	'dose-dependent increase of eCB serum levels' reported, but data not shown[d]	[217]
ASP3652 (placebo) Sponsor: Astellas Pharma Europe B.V.	NCT01613586; randomized double-blind adaptive parallel assignment	Women with bladder pain syndrome / interstitial cystitis; 50, 150 or 300 mg b.i.d.	Change from baseline in Mean Daily Pain (MDP) after 12 weeks of treatment	For all three dose regimes ($N = 49$–90), no significant difference in the change in baseline to that seen with placebo ($N = 75$) was found	Dose-dependent 'increase of endocannabinoid plasma levels, up to a maximum increase of approximately four times their baseline level' reported, but data not shown	[218]

Table 4 (*Continued*)

FAAH inhibitor (comparator)	ClinicalTrials.gov protocol; trial design	Disorder; Dose regime	Registered primary aim	Outcome of primary aim	Comments	Ref.
ASP8477 (placebo) Sponsor: Astellas Pharma Europe B.V.	NCT02065349; screening-, single-blind titration and maintenance-, double-blind randomized treatment ('dbrt')- and follow-up periods	Painful diabetic peripheral neuropathy or postherpetic neuralgia; 20 or 30 mg b.i.d.[e]	Change in mean of 24-hour average pain intensity, Numeric pain rating scale (NPRS) from baseline to the last three days of the 21-day dbrt period	No significant difference in the primary endpoint was seen between the ASP8477- ($N = 31$) and placebo- ($N = 32$) treated patients who completed the double-blind part of the study	During single-blind period, mean plasma AEA levels increased from 0.45 ± 0.18 ng mL^{-1} (SD, $N = 113$) at baseline to 2.96 ± 0.68 ng mL^{-1} ($N = 110$) 4 h post-dose on day 14. PEA and OEA levels were also increased. 59% (ASP8477) and 41% (placebo) of the patients used concomitant pain medication during the dbrt period	[219]
V158866 (placebo) Sponsor: Vernalis (R&D) Ltd	NCT01748695 randomized double-blind crossover	Neuropathic pain due to spinal cord injury. 450 mg once daily	Mean Pain Intensity (NRS) over last 7 days of treatment compared to placebo (total treatment time 4 weeks)	No significant difference between active treatment and placebo was seen ($N = 25$; 14 placebo then V158866; 11 V158866 then placebo)		[220]
SSR411298 (placebo) Sponsor: Sanofi	NCT01439919; randomised double-blind parallel assignment	Adjunctive treatment for persistent cancer pain 200 mg once daily	Numeric Rating Scale (NRS) compared to placebo after 4 weeks of treatment	No results posted, other than that the actual enrolment into the study was 5 individuals	The study was terminated 'due to strategic reasons'	[221]

Table 4 (*Continued*)

FAAH inhibitor (comparator)	ClinicalTrials.gov protocol; trial design	Disorder; Dose regime	Registered primary aim	Outcome of primary aim	Comments	Ref.
IW-6118 (placebo) (naproxen) Sponsor: Ironwood sponsor does not mention the compound on their website, so presumably it has been dropped	Pharmaceuticals, Inc. [222]	NCT01107236; randomized double-blind parallel assignment	Otherwise healthy patients undergoing third molar extraction. Dose not specified	Safety assessments after single dose[f]	No results posted, other than that the actual enrolment into the study was 90 individuals	The

[a]Studies [149,217–220] also reported safety data: in general, the compounds were well-tolerated by the patients.
[b]Western Ontario and McMaster Universities Osteoarthritis Index.
[c]National Institutes of Health-Chronic Prostatitis Symptom Index.
[d]The authors also reported a *post hoc* subgroup analysis indicating that micturition outcomes (reduction in voids per 24 h) were improved in all the five dose regimes compared to placebo in a subset of patients with increased voiding frequency (see [223] for a critical discussion with respect to *post hoc* subgroup analyses).
[e]These are for the double-blind period. Patients who did not tolerate the higher dose were given the lower dose.
[f]'Efficacy will be assessed in an exploratory manner'.

lipoxygenases and CYP450 oxidases to produce biologically active compounds [80,81,121,122]. Most work on these has been undertaken on the COX-2-derived prostaglandins (PGs), the PG-ethanolamides (PG-EAs, prostamides) and the PG glyceryl esters, which show both pro- and anti-inflammatory activities (review see [123]). Thus, for example, Gatta *et al.* [124] showed that kaolin/λ-carrageenan-induced inflammation of the knee resulted in increased levels of $PGF_{2\alpha}$-EA in the rat spinal cord, and that in control animals, the spinal administration of this prostamide increased the firing of dorsal horn nociceptive neurons. In contrast, PGD_2-GE, but not PGD_2, decreases the mechanical hyperalgesia and oedema produced by intraplantal injection of λ-carrageenan in mice [125].

Translation, or lack thereof, of FAAH inhibitors to the clinic for the treatment of pain

Preclinical studies with FAAH inhibitors showed great promise with respect to pain, since different classes of compounds showed efficacy in animal models of persistent, visceral, inflammatory and/or neuropathic pain [126–131] (for details of all the studies in pain models with FAAH inhibitors up to 2015, see Tables 1-5 of [132]), without producing THC-like behaviours [110,133], substitution for THC in drug discrimination tests [109], or reinforcing behaviour in squirrel monkeys trained to self-administer THC or cocaine [134]. A molecular genetic study associating a *FAAH* gene polymorphism with pain sensitivity [135] and a recent case report of a woman with pain insensitivity who had a heterozygous microdeletion downstream from the 3' end of *FAAH* [136] also tie in FAAH with pain.

Table 5. *Possible reasons for the lack of efficacy of FAAH inhibitors in clinical trials of pain*

Hypothesis	Potential solutions
FAAH is not a suitable target in the pain types investigated	MAGL inhibitors
Suboptimal increase in AEA due to alternative catabolic pathways or due to deleterious effects of products of such pathways	Dual-action compounds inhibiting FAAH and the additional pathway (NAAA, FAAH-2, COX-2) or the receptor for the downstream product ($PGF_{2\alpha}$-EA)
Increased AEA levels alone are not sufficient to alleviate pain, and other members of the extended eCB system need also to be potentiated sufficiently	NAAA or MAGL inhibitors ± FAAH inhibitors
Beneficial effects of the increased AEA concentrations are negated by effects at TRPV1 receptors	Dual-action FAAH inhibitor / TRPV1 antagonists

The initial clinical studies with FAAH inhibitors in healthy volunteers indicated that compounds such as PF-04457845, V158866 and JNJ-42165279 were well tolerated, increased plasma AEA and other NAE levels, and, in the case of PF-04457845, did not produce cognitive effects [137–139] consistent with the pre-clinical studies. JNJ-42165279 was also found to produce a profound occupancy of brain FAAH and to increase AEA levels in the cerebrospinal fluid [139]. However, the Phase 2 clinical studies with FAAH and pain have been a disappointment, with several studies showing a negative outcome (Table 4). This somewhat depressing picture was compounded in 2016 when a Phase I multiple ascending dose trial of Bial's FAAH inhibitor BIA 10-2474 resulted in severe neurological adverse effects and one death [140]. Such a tragedy was unexpected, given that all other FAAH inhibitors are well tolerated by patients (as had lower doses of BIA 10-2474 been in previous cohorts). These severe adverse effects are likely related to off-targets of BIA 10-2474 and/or its metabolite(s) [141,142], possibly coupled to an overly rapid sequential dosing protocol [143]. When the tragedy unfolded, the US Food and Drug Administration halted ongoing clinical trials with FAAH inhibitors, but later concluded that 'based on the available information ... BIA 10-2474 exhibits a unique toxicity that does not extend to other drugs in the class, called fatty acid amide hydrolase (FAAH) inhibitors'. [144].

Alternative approaches to harness FAAH inhibition for the treatment of pain

The above discussion would suggest that FAAH inhibition *per se* is not a useful approach to treat pain despite the promising preclinical data. The predictive validity of the standard pain models has

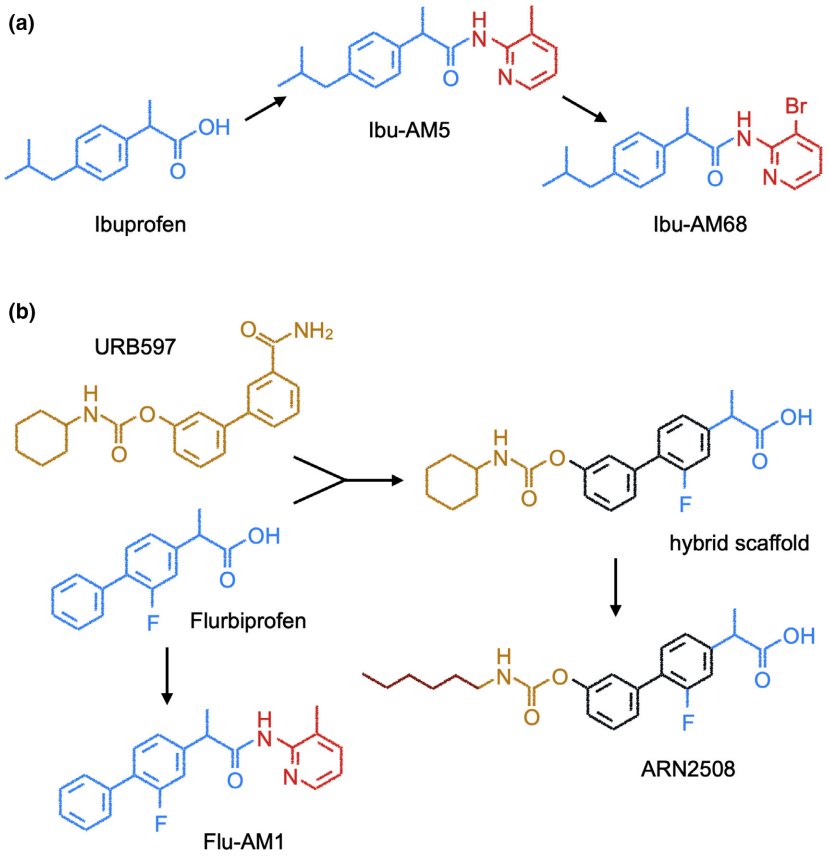

Fig. 4 *Structures of dual-action FAAH / COX inhibitors based upon a) ibuprofen and b) flurbiprofen [150–153].*

been discussed earlier with respect to CB_2 receptor agonists, and it is notable that in a model of non-evoked pain (burrowing behaviour in a monosodium iodoacetate model of osteoarthritis at a time-point where the pain is mainly mediated by inflammation), the FAAH inhibitor PF-04457845 was not effective, in contrast to ibuprofen, celecoxib and an antibody to tumour necrosis factor-α [145]. Animal models aside, Table 5 summarizes some possible explanations as to why FAAH inhibitors *per se* were ineffective in clinical pain as a way of introducing possible ways forward.

The simplest explanation is that FAAH does not engage the target sufficiently at the doses used. Two of the clinical trials with FAAH inhibitors in pain reported increases in plasma AEA levels (Table 4), but that does not prove target engagement elsewhere. *In extremis*, the body may already have undertaken locally what the FAAH inhibitor was meant to do. This is in admittedly in the realm of speculation, but FAAH expression and activity in human lymphocytes is decreased following 24 h *in vitro* treatment with either interleukin-12 or interferon-γ and increased with interleukins 4 and 10 [146], so the enzyme is clearly sensitive to the inflammatory environment. An increased AEA concentration is not a universal response to FAAH inhibition: for example, intraplantally administered URB597 does not increase levels of AEA in the hind paw of rats with spinal nerve ligation whereas an increase is seen for sham-operated rats [147].

A variation of the above relates to the alternative catabolic pathways shown in Fig. 2, namely that AEA levels are increased as a result of FAAH inhibition, but the increase is insufficient due to its removal by other enzymes. In this respect, Benson *et al.* [148] modelled the data from the PF-04457845 clinical trial of Huggins *et al.* [149]

and suggested that plasma AEA time curve following PF-04457845 treatment, which included a long plateau region, could not 'be adequately described without evoking an additional fatty acid amide hydrolase (FAAH)-independent clearance process'. Of course, the model is only as good as the assumptions made, but it motivates consideration of blockade of other AEA-catabolic enzymes in addition to FAAH. Benson *et al.* [148] suggested that NAAA could be the enzyme responsible for removal of AEA following FAAH inhibition by PF-04457845. An alternative could be that inhibition of both FAAH and FAAH-2 is required in humans. PF-3845, a potent and highly selective FAAH inhibitor with a structural similarity to PF-04457845, does not inhibit FAAH-2 (IC_{50} value > 10 μmol L^{-1}) [127]. Since rats and mice do not express FAAH-2 [103], this pathway would not be operative in the animal pain models.

COX-2 may also provide an important alternative pathway for AEA following FAAH inhibition in the patients investigated in the clinical trials, not least since it is induced in inflammatory conditions. FAAH - COX dual-action inhibitors have been designed [150–153], based upon increasing the modest FAAH inhibitory potencies of the profen class (ibuprofen [154], flurbiprofen) of non-steroidal anti-inflammatory drugs (NSAIDs) whilst retaining their COX inhibitory potency (Fig. 4). In experimental animals, two of the compounds (Ibu-AM5 and ARN2508) are biologically active *in vivo*, but do not cause gastric ulcers when given acutely, in contrast to the NSAIDs ibuprofen and ketorolac [152,155]. This may be due to their FAAH-inhibitory properties, since FAAH inhibitors (or genetic deletion of FAAH) protect against NSAID-induced acute gastric ulcers in experimental animals [131,156]. Ibu-AM5 and (*R*)-Flu-AM1 also show an interesting property first described for (*R*)-profens [157], namely that they inhibit COX-2-catalysed cyclooxygenation of eCBs more potently than the corresponding cyclooxygenation of arachidonic acid [151,158]. Most recently, ATB-352, a hydrogen sulphide-releasing analogue of ketoprofen that does not cause gastrointestinal ulceration [159] has been shown potently to inhibit FAAH and to reduce mechanical allodynia in a model of postoperative pain in a CB_1-receptor mediated manner [160]. Compounds inhibiting FAAH and the $PGF_{2\alpha}$-EA receptor have also been described [161] (*q.v.* the pro-algesic effects of $PGF_{2\alpha}$-EA [124]).

Another potential explanation for the poor outcomes in the clinical trials with FAAH is that potentiation of 2-AG rather than AEA may be more important in some pain syndromes. Like FAAH inhibitors, selective MAGL inhibitors have been shown to produce potentially beneficial effects in models of visceral, inflammatory and neuropathic pain (see Tables 1-5 of [132]), but to my knowledge clinical data for MAGL inhibitors is not yet available with respect to pain. Additionally, compounds inhibiting both MAGL and FAAH could be considered, although it is hard to see the advantage of such compounds *vs.* THC, given that they produce similar behavioural effects at least in animal models [109,110].

An alternative possibility is that in humans, the pain regulatory response is a combination of effects produced by both AEA and PEA, since these are both produced at the same time (see above), and since PEA has anti-inflammatory and analgesic properties (see [86]). In this case, the argument would be that FAAH inhibition increases NAE levels, but that the increase in PEA levels is insufficient to mitigate the pain. Selective NAAA inhibitors have been described and have been shown to produce beneficial effects in animal models of inflammatory pain (for an example, see [162]) and so it would clearly be of interest to investigate whether the combination of an FAAH and an NAAA inhibitor is beneficial in human pain.

The final suggestion listed in Table 5 can be linked to an observation using cultured rat primary sensory neurons that in inflammatory conditions, the efficacy of AEA for TRPV1 is increased [163]. This raises the possibility that in the clinical trials, the beneficial effects produced by increasing AEA concentrations secondary to FAAH inhibitor are negated by TRPV1 effects mediated by this eCB or other NAEs such as OEA [149]. This would motivate clinical studies of FAAH inhibitors together with TRPV1 antagonists or alternatively dual-action compounds with FAAH inhibitory and TRPV1-antagonistic actions. Such molecules have been designed [164,165] and shown to be active in animal pain models [166,167]. Inhibition of FAAH and soluble epoxide hydrolase may also be a useful combination [168] and molecules inhibiting both enzymes have been described [169].

Other potential indications for FAAH inhibitors

The observations that the adverse effects profile of the CB_1 receptor inverse agonist rimonabant had

an unacceptable incidence of anxiety and depression [73] raises the possibility that FAAH inhibitors, by raising endogenous AEA-mediated tonus, could have useful anti-anxiety and antidepressive properties. Indeed, potentially useful effects of FAAH inhibitors in a number of different animal models of anxiety, depression and compulsive behaviour have been reported (review see [170]).

With respect to treatment of major depressive disorder, two studies have been registered at ClinicalTrials.gov, one with SSR411298 (Sanofi, ClinicalTrials.gov NCT00822744, double blind, placebo-controlled, 8-week treatment in elderly patients with escitalopram as comparison), and one with JNJ-42165279 (Janssen Research & Development, LLC, ClinicalTrials.gov NCT02498392, double-blind placebo-controlled study in patients with major depressive disorder with anxious stress). To my knowledge, results of these studies have not yet been published in peer-reviewed journals, although Mandrioli and Mercolini [171] reported that SSR411298 was not more effective than placebo in the NCT00822744 trial and that its development for this indication has been discontinued[3].

More information is available concerning the potential of FAAH inhibitors for treatment of social anxiety and post-traumatic stress disorder. With respect to the former, a double-blind placebo-controlled study of JNJ-42165279 in social anxiety disorder has just been published [172]. In this study JNJ-42165279 or placebo was given for 12 weeks, and the primary outcome measure was change from baseline in the Liebowitz Social Anxiety scale. No significant difference was seen in the primary outcome measure, although a secondary outcome, the percentage of patients with a ≥ 30 improvement in baseline, was significantly higher than placebo (44% vs 24%, $P = 0.04$). On the basis of measurement of trough plasma concentrations of the drug and plasma AEA concentrations (which were highly correlated), the authors argued that the dose used (25 mg once daily) might have not been sufficient and they intend to investigate a different dose regime (25 mg b.i.d.) [172].

With respect to post-traumatic stress disorder (PTSD), no ongoing trials are listed on ClinicalTrials.gov (search word 'FAAH') as of November 2020, but an interesting double-blind, placebo-controlled study on the effects of PF-04457845 (4 mg day^{-1} for 10 days) on fear extinction and stress responses in healthy individuals has been published [173]. On days 9 and 10 after the start of treatment, which for PF-04457845 was sufficient to increase plasma AEA levels by an order of magnitude, the patients undertook a series of behavioural tests including eyeblink responses to a 50 ms burst of white noise and an aversive sound of nails across a chalkboard as unconditioned stimulus, and mental arithmetic tests with 'negative socioevaluative feedback'. An affective image task was undertaken before and after the stress tests and the control tasks [173]. PF-04457845 did not affect acquisition of conditioned fear but promoted recall of fear extinction memory when tested on the second day. The negative affect understandably produced by the stress paradigm was also attenuated for the negative images in the image bank used. These data raise the possibility that FAAH inhibition may be a potentially useful treatment for at least some of the symptoms of PTSD. The authors of [172] also reported that they are 'initiating trials in PTSD with increased doses' of JNJ-42165279.

FAAH inhibitors may also be useful for cannabis use disorder. Thus, PF-04457845 (4 mg day^{-1}) was found to reduce cannabis withdrawal symptoms and subsequent cannabis use (as assessed by self-reported cannabis use and measurement of the urinary levels of the THC metabolite THC-COOH) in men with cannabis use disorder, leading to the authors to conclude that PF-04457845 'might represent an effective and safe approach for the treatment of cannabis use disorder' [174].

An MAGL inhibitor, LuAG06466 is early on in its clinical development, also with PTSD and other neurological/psychiatric disorders as potential indications [175] (for a review on the potential of agents affecting the eCB system as treatments for neurological disorders, see [10]). I presume LuAG06466 is the same compound as ABX-1431 [176] which had undergone some initial trials in patients with Tourette Syndrome or Chronic Motor

[3]The link to a press release by Sanofi given in this paper is no longer active, but the press release stating that "Two projects in Phase II were discontinued. Data ... on SSR411298 in major depressive disorders, did not support progression to Phase III trials" can be found at http://www.news.sanofi.us/press-releases?item=118522 (URL checked 12 November 2020).

Tic Disorder [177], and a study to determine whether the compound produces tolerance in patients with neuropathic pain [178].[4] The latter is an important consideration given that the first selective MAGL inhibitor, JZL184, produced behavioural tolerance and down regulation of CB_1 receptors in mice upon repeated administration [179].

Conclusions

The present article has aimed to present the current state of the art of drug development in the eCB field. Despite the setbacks in the clinical trials for pain with CB_2 receptors and FAAH inhibitors, the area remains active, and of necessity, I have not taken up potential indications in areas such as migraine, Parkinson's disease, multiple sclerosis, inflammatory bowel disease and cancer (reviews, see [10,31,180–182]) or with respect to the treatment of cannabis use disorder or cannabis-induced hyperemesis syndrome [183,184]. Similarly, the increasing use of markers of the eCB system in PET studies [139,185] is a fascinating area of research whereby CB_1 receptor, FAAH and MAGL ligands have been adopted to probe the eCB system in the human brain. It is to be hoped that the rate of discoveries made in the quarter of a century or so since the identification of the eCBs AEA and 2-AG will continue over the next twenty-five years and, not least, result in the clinical use of novel drugs modulating the eCB system.

Acknowledgments

As the author heads towards his retirement in 2021, he would like to take this opportunity to thank all those researchers in the endocannabinoid field whose work has been a source of inspiration over the years.

[4]Lundbeck has recently acquired Abide Therapeutics, who developed ABX-1431. PF-04457845 is also on the move: Pfizer licenced the compound to its spin-off company SpringWorks Therapeutics who in turn have licenced the global rights of the compound to Jazz Pharmaceuticals "for the potential treatment of PTSD" (https://www.prnewswire.com/news-releases/jazz-pharmaceuticals-acquires-springworks-therapeutics-faah-inhibitor-program-301159303.html, URL checked 16 November 2020).

Author contribution

Christopher Fowler: Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Visualization (lead); Writing-original draft (lead); Writing-review & editing (lead).

Conflict of interest statement

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