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Pain and beyond: fatty acid amides and fatty acid amide hydrolase inhibitors in cardiovascular and metabolic diseases

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Fatty acid amide hydrolase (FAAH) is responsible for the hydrolysis of several important endogenous fatty acid amides (FAAs), including anandamide, oleoylethanolamide and palmitoylethanolamide. Because specific FAAs interact with cannabinoid and vanilloid receptors, they are often referred to as 'endocannabinoids' or 'endovanilloids'. Initial interest in this area, therefore, has focused on developing FAAH inhibitors to augment the actions of FAAs and reduce pain. However, recent literature has shown that these FAAs – through interactions with unique receptors (extracellular and intracellular) – can induce a diverse array of effects that include appetite suppression, modulation of lipid and glucose metabolism, vasodilation, cardiac function and inflammation. This review gives an overview of FAAs and diverse FAAH inhibitors and their potential therapeutic utility in pain and non-pain indications.

Introduction

Fatty acid amide hydrolase (FAAH) is an integral membrane protein and is the primary enzyme responsible for catabolism of the fatty acid amide (FAA) family of endogenous signaling lipids. These FAAs are often referred to as 'endocannabinoids' because they function as agonists of the cannabinoid receptors CB1 and CB2. They are also referred to as 'endovanilloids' because of their effects on vanilloid receptors (members of the transient receptor potential [TRP] family of calcium channels, e.g. TRPV1). The endogenous FAAs have been shown to modulate these receptors and elicit a wide variety of activities. The next sections include detailed discussion on the function and roles of FAAs, including the potential therapeutic utility of FAAH inhibition in different disease settings (including pain, related central nervous system disorders, inflammation, diabetes/obesity and cardiovascular diseases).

Endogenous FAAs

Classification and structure

Endogenous FAAs can broadly be categorized into the chemical classes shown in Table 1. Although there are several subclasses of FAAs, the *N*-acylethanolamines (NAEs) have been most studied and would be the major focus of discussion in this review. The structure and function of other classes of FAAs have been summarized well in a recent review [1].

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Dr. Sivaram Pillarisetti is the vice president of Research at Dr. Reddy's. He has been conducting research in the therapeutic areas of cardiovascular and metabolic diseases for over 18 years. Prior to Reddy US Therapeutics/Dr. Reddy Laboratories, Dr. Pillarisetti had held faculty appointments at Columbia University and the North Shore-Long Island Jewish Health System in New York. His areas of expertise include diabetes, atherosclerosis, inflammation and therapeutic angiogenesis. He is the author of over 45 research papers and review articles and an inventor on more than a dozen issued patents. Dr. Pillarisetti had been an investigator of the American Heart association (1996–99) and is a current member of the American Diabetes association and a fellow of the American Heart Association.

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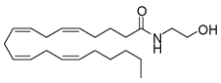
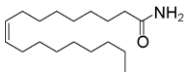
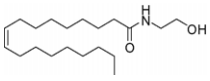
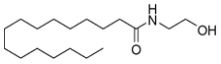
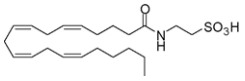
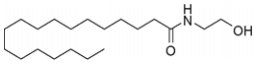
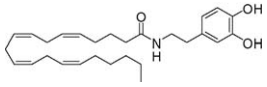
TABLE 1

Fatty acid amide families

N-Acylethanolamine (NAE)		Primary fatty acid amide	
AEA (*N*-arachidonoyl ethanolamide or anandamide)		Oleamide; (9*Z*-octadecenamide)	
OEA (oleoylethanolamide)		*N*-acylamino acids (NAA)	
PEA (*N*-palmitoyl ethanolamide)		NAT (*N*-arachidonyl taurine)	
SEA (*N*-stearoyl ethanolamide)		*N*-acyldopamine	
		NADA (*N*-arachidonoyl dopamine)	

Several NAEs have been identified in mammalian tissues. In brain, the most abundant are *N*-palmitoyl-, *N*-stearoyl- and *N*-oleoylethanolamides, which total ~75% of brain NAEs [1]. The less-abundant NAEs found in the brain include anandamide (*N*-arachidonoyl ethanolamide, or anadamide) and *N*-linoleoyl-, *N*-linolenoyl-, *N*-dihomo-γ-linolenoyl- and *N*-docosatetraenoyl-ethanolamides. The NAEs are widespread in the peripheral tissues [1–10].

Anadamide was discovered in 1992 by Devane and colleagues [3] and named after the Sanskrit word *ananda*, which means 'bliss or delight'. Anandamide acts as an endogenous agonist for the central cannabinoid receptor (CB1) and modulates several neurobehavioral processes, including pain, feeding and memory [11–13]. Like anandamide, other more abundant NAEs, such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), also regulate pain and feeding [14,15]. These fatty amide levels are elevated in response to pain stimuli (electrical and chemical) and are thought to produce analgesia and dampen the spinal and thalamic neuronal responses to noxious stimuli [16].

Although much of the early work focused on pain and related neurological effects, the NAEs demonstrate a wide spectrum of activities with potential utility in modulating cardiovascular and metabolic disorders.

Endocannabinoid biosynthesis

Biosynthesis of endocannabinoids such as anandamide has been studied well [17,18]. Anandamide is believed to originate from hydrolysis of *N*-acyl phosphatidylethanolamine (NAPE, A1) by phospholipase D (PLD). The precursor A1 is formed by *N*-arachidonoylation of phosphatidylethanolamine using Ca^{+2}-dependent *N*-acyltransferase (NAT). The two-step process involving NAT and PLD-mediated hydrolysis has been implicated in biosynthesis of most endocannabinoids. Anandamide synthesis continues to occur in NAPE–phospholipase-deficient animals, however, suggesting that alternate enzymes might also play a part in the generation of FAAs [19]. NAPE–PLD deletion seems to have a dramatic impact on the levels of long-chain saturated NAEs, but biosynthesis of polyunsaturated NAEs, including anandamide, is unaffected by NAPE–PLD inactivation; thus, their biosynthesis seems to be controlled by other enzymes. It appears that the NAPE–PLD-independent pathway might be mediated by another PLD enzyme because significant NAPE–phospholipase activity was measured in brains from NAPE–$PLD^{-/-}$ mice, especially when assays were run in the absence of calcium. It remains to be seen whether there is a calcium-independent NAPE–phospholipase activity that is essential for anandamide synthesis. Immediately after synthesis, the FAAs are released from cells because no mechanism exists for their storage.

Molecular targets for endocannabinoids

Anandamide can interact with both CB1 and CB2 [1,2]. The effects of anandamide are mediated largely by its binding to the CB1 receptors (K_d = 80 nM) and CB2 receptors (K_d ~500 nM). With the exception of anandamide, *N*-dihomo-γ-linolenoylethanolamine and *N*-docosatetraenoylethanolamine, other NAEs do not bind to the CB1 and CB2 receptors [1,2]. Other receptors that anandamide is known to interact with an affinity of 1 μM or less are listed in Table 2. The list includes voltage- and ion-gated channels and other receptors. Because of its lower affinity to non-CB1 receptors, contribution of these receptors to anandamide effects *in vivo* is unclear. When calcium mobilization is induced, endogenous anandamide release can activate TRPV1 receptors, and this effect appears to precede activation of CB1 receptors [20].

Several NAEs, primary FAAs and *N*-acyl taurines interact with vanilloid receptor TRPV1 [4,10,21,4,22–24]. The C18 unsaturated NAEs such as *N*-linolenoylethanolamine (18:3 NAE), *N*-linoleoylethanolamine (18:2 NAE) and *N*-oleoylethanolamine (18:1 NAE) – but not saturated *N*-stearoylethanolamine (18:0 NAE) – activate TRPV1 channels at submicromolar concentrations [4]. Molecular modeling studies have revealed a low-energy cluster of U-shaped

TABLE 2

Other receptors that anandamide (AEA) interact at concentration of 1 μM or less[a]

Target channel	End-point measurement	Effect	Concentration of AEA
Voltage-gated ion channels			
N-type Ca^{2+}	Ion current	Inhibition	1–10 μM
T-type Ca^{2+}	Ion current	Inhibition	IC_{50} = 330 nM–4 μM
Leak K^+ channels TASK	Ion currents	Inhibition	IC_{50} = 0.7 μM
***Shaker* Kv1.2 K^+**	Ion current	Inhibition	IC_{50} = 2.7 μM
Ca^{2+}-activated K^+ channels (BK)	Ion current	Potentiation	0.3–3 μM anadamide
Delayed rectifier K^+	Ion current	Inhibition	IC_{50} = 0.6 μM
Delayed rectifier K^+ Kv3.1	Ion current	Inhibition	$IC_{50} \approx$ 80 nM
Kv4.3	Ion current	Inhibition	$IC_{50} \approx$ 80 nM
Ligand-gated ion channels			
$5\text{-}HT_3$	Ion current	Inhibition	IC_{50} = 130 nM–3.7 μM
α-7 nACh	Ion current	Inhibition	IC_{50} = 230 nM
GlyR	Ion current	Inhibition and potentiation	IC_{50} = 200–300 nM
NR1A NMDA	Ion current	Potentiation	0.1–1 μM
TRPV1 receptors	Ion currents, Ca^{2+} measurements	Activation	IC_{50} = 0.7–10 μM
TRPV4 receptors	Ion currents	Activation	1–10 μM
Other channels and cellular events			
5-HT receptors	Radioligand binding	Inhibition	IC_{50} = 1–10 μM
Ca^{2+} release	Ca^2+ measurements or contractures	Increase	1–30 μM
Arachidonic acid release	Biochemical assays	Increase	≥1 μM
PLC and PLD	Enzyme assay	Activation	≥1 μM

[a] Oz, M. (2006) Receptor-independent action of cannabinoids on cell membranes: focus on endocannabinoids. *Pharm. Therap.* 111, 114–144 (and references cited therein).

unsaturated NAE conformers, sharing several pharmacophoric elements with capsaicin, a well-known ligand of TRPV1 [4].

NAEs, including anandamide and OEA, can also interact with nuclear peroxisome proliferator-activated receptor (PPAR) α and γ (PPARα, K_d = 20 μM and PPARγ, K_d = 10 μM). Activation of PPARα, in part, seems to mediate the biological effects of OEA [24–26]. *N*-Oleoylethanolamine can bind to the G-protein-coupled receptor (GPCR) GPR119 [27]. Another GPCR, GPR55, has also been shown to interact with certain FAAs, although it is not clear whether any biological effects of FAAs are mediated by this receptor [28]. It has been suggested that at least some of the activities of the NAEs *N*-palmitoylethanolamine, *N*-oleoylethanolamine and *N*-stearoylethanolamine result from the 'entourage effect' (i.e. either cellular levels of anandamide are stabilized or increased because NAEs compete with anandamide for enzymatic degradation [29] or NAEs, such as PEA, can potentiate anandamide actions on TRPV1, possibly by an allosteric mechanism [30]).

Endogenous NAEs: role in disease states

The biological effects of FAAs are summarized in Fig. 1. FAAs are produced locally in many tissues and their levels are deregulated under different pathological conditions. In the following sections, we will review their regulation and possible metabolic effects.

CNS effects (pain, anxiety and sleep disorders) of FAAs

As discussed above, anandamide acts as an endogenous agonist for the central CB1 receptor and capsaicin receptor TRPV1 and modulates several neurobehavioral processes, including pain, feeding and memory [12–14]. Anandamide–CB1 interactions result in the activation of G proteins, particularly those of the Gi/o family, resulting in inhibition of adenylyl cyclase and regulation of ion currents, culminating in analgesic response. Like anandamide, OEA and PEA regulate pain and feeding [15]. PEA exerts potent analgesic effects in experimental models of visceral, neuropathic and inflammatory pain by acting via several possible mechanisms (see below).

Anandamide is also involved in the regulation of body temperature, locomotion, feeding and anxiety. OEA and the primary FAA oleamide (OA) both induce sleep. Most of the anandamide effects are mediated by the cannabinoid receptor CB1, although it is conceivable that non-CB1 receptors (Table 2) might contribute to some of the effects.

Anti-inflammatory effects of FAAs

Although CB1 is the primary receptor in brain, both CB1 and CB2 receptors are present in peripheral tissues and have been shown to play a part in controlling inflammation and immune cell function [31,32]. FAAs are effective in different animal models of inflammation. In models of colon inflammation, increasing anandamide levels blocks development and symptoms of inflammatory bowel disease significantly [33]. The anti-inflammatory effects of PEA have been studied extensively in animal models [34–38]. PEA behaves like an autacoid, modulating the mast-cell response to inflammation, and has been shown to be effective in both developing and curative models of inflammation. PEA reduces substance-P-induced mast-cell degranulation and plasma extravasation [37]. It also reduces carrageenan-, formalin- and dextran-induced edema and carrageenan-induced hyperalgesia [36–38]. Unlike anandamide, the mechanism of PEA anti-inflammatory effects is not completely understood. The involvement of CB1 and CB2 cannabinoid receptors seems unlikely because PEA does not bind to these receptors with high affinity. Some studies reported reversal of PEA effects by selective CB2 receptor antagonist SR144528 [33], whereas others showed no effect [38]. Other possible mechanisms for the anti-inflammatory effect of PEA include a decrease in cyclooxygenase-2 (Cox2) and inducible nitric

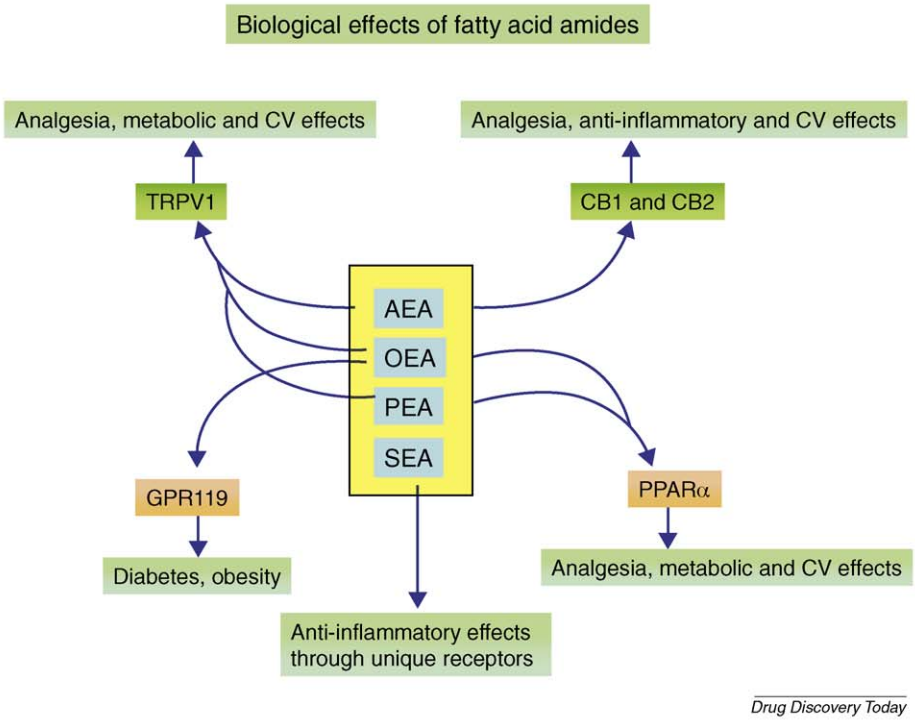


FIGURE 1

Biological effects of fatty acid amides (FAAs). Focus on NAEs. The receptors that FAAs interact with and potential therapeutic implications of these interactions in pain, metabolic and cardiovascular indications are presented.

oxide synthase (iNOS) expression [38] or potentiating the activity of endogenous anandamide (entourage effect) by competing with its degradation by FAAH [39].

Anti-inflammatory effects of several NAEs have been demonstrated in immune cells through activation of the CB2 receptor. For example, in peripheral mononuclear cells, both anandamide and PEA inhibited interleukin (IL)-6 and IL-8 production at low nanomolar concentrations (3–30 nM) [40]. In addition, anandamide inhibited the production of tumor necrosis factor alpha (TNFα), interferon-gamma, IL-4 and p75 TNFα soluble receptors at low micromolar concentrations. In cultured endothelial cells, anandamide dose dependently decreases the TNFα-induced adhesion molecule expression and adhesion of monocytes. In lipopolysaccharide (LPS)-induced bronchopulmonary inflammation in mice, both anandamide and PEA reduced TNFα in bronchoalveolar lavage fluid [41].

More recent studies suggest that PEA and other FAA effects might be mediated, in part, by the activation of PPARα [37], a nuclear transcription factor present in multiple tissues. PEA activates PPARα *in vitro* with an EC_{50} of 3.1 μM and induces the expression of PPARα mRNA when applied topically to mouse skin. In carrageenan-induced paw edema and phorbol ester-induced ear edema models, PEA attenuates inflammation in wild-type mice but not in mice deficient in PPARα [37,42]. This contrasts OEA's analgesic effects, which do not seem to depend on PPARα but require participation of glutamatergic transmission [15] because the effects are blocked by MK-801, a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, one of the three known glutamate receptors.

Like PEA, *N*-stearoyl ethanolamine (SEA) exerts marked anti-inflammatory properties *in vivo*. Unlike OEA or PEA, SEA effects are fully reversed by capsazepine, a competitive antagonist of the TRPV1 receptor [43].

Metabolic effects (food intake, obesity and diabetes) of FAAs

The gastrointestinal (GI) tract has an important role in the regulation of food intake and energy balance. GI signals can limit ingestion and regulate feeding behavior [44,45]. FAAs are produced within the GI system and the levels are inversely correlated to feeding [46,47]. FAAs are physiological regulators of intestinal motility and food intake via regulation of gastric emptying. There are high amounts of anadamide in the intestines [46,47]. In mice fed with standard diet or high-fat diet, unsaturated NAEs, anandamide and OEA, but not PEA, reduced gastric emptying [48,49]. Feeding increases the levels of unsaturated fatty acid *N*-ethanolamides without affecting those of saturated fatty acid ethanolamides such as PEA. Feeding-induced FAA formation is accompanied by enhanced accumulation of FAA-generating NAPEs, increased activity and expression of NAPE–PLD, and decreased activity and expression of FAAH [50].

Several mechanisms have been proposed for FAA-induced changes in gastric emptying. This effect of anandamide was counteracted by the CB1 receptor antagonist rimonabant but not by the CB2 receptor antagonist SR144528 or by the TRPV1 antagonist 5′-iodoresiniferatoxin [48]. The FAAH inhibitor *N*-arachidonoyl-5-hydroxytryptamine also reduced gastric emptying [48], suggesting that endogenous FAA levels exert an effect on gastric emptying.

Although CB1 might play a part in anandamide-induced satiety, the mechanisms behind OEA effects seem complex and controversial. Early studies suggested a PPARα mechanism for OEA activity [51]. OEA produces satiety and reduces body weight gain in wild-type mice but not in mice deficient in PPARα [49]. In the small intestine of wild-type (but not PPARα-null) mice, OEA regulates the expression of several PPARα target genes and represses inducible nitric oxide synthase, an enzyme that might contribute to feeding stimulation. It is conceivable that PPARα activation in sensory afferent neurons transduces information to specific areas of the brain. Later studies further confirmed PPARα mechanism and OEA analogs with potent PPARα activity reduced food intake in mice [52]. By contrast, in studies by Aviello *et al.* [49], the effect of OEA was unaffected by CB1/CB2 antagonists (rimonabant/SR144528), TRPV1 antagonist (5′-iodoresiniferatoxin) or PPARα antagonist (MK886). The reasons for the discrepancy are not clear.

OEA and PEA are also produced locally in adipose tissue. OEA reduces visceral fat mass [51–53] and produces several peripheral effects. Systemic administration of OEA stimulates lipolysis and produces a rapid elevation of the circulating levels of nonesterified fatty acids and glycerol and a decrease in triacylglycerol content in the epididymal fat and in the liver. [53]. OEA also stimulates fatty acid oxidation in rat soleus muscle and ketogenesis in rat hepatocytes [53]. In the liver, OEA regulates the expression of genes involved in lipid metabolism, such as PPARα and some of its targets (e.g. FAT/CD36, liver fatty-acid-binding protein and uncoupling protein-2) [52]. PEA levels are decreased during adipose differentiation; leptin and PPARγ activation might contribute to this downregulation [54]. In diet-induced obesity models, OEA and PEA levels are significantly downregulated. Similarly, in obese patients, subcutaneous fat contained significantly lower levels of PEA. These observations suggest that downregulation of FAA levels might contribute not only to fat accumulation but also to increased inflammation in adipose. Elevation of FAAs has multiple beneficial effects, reducing fat content in adipose while promoting fat utilization in muscle and liver.

OEA and other NAEs also possess potential antidiabetic effects. OEA and PEA are produced by cultured pancreatic cells and their levels are regulated by hyperglycemia and insulin [54]. In the blood of nonobese type 2 diabetes patients, PEA and OEA levels are increased significantly compared with age-, BMI- and gender-matched normoglycemic subjects. OEA can promote glucose-dependent activation of GPR119, a GPCR present on pancreatic islet cells and intestinal endocrine cells. OEA binds and activates GPR119, thereby increasing intracellular cAMP, leading to increased glucose-dependent insulin secretion from pancreatic β cells and incretin glucagon-like peptide-1 (GLP-1) secretion from enteroendocrine cells. In various animal models of type 2 diabetes and obesity, OEA lowered blood glucose without hypoglycemia, slowed diabetes progression and reduced food intake and body weight [26,55].

Whereas FAAs in the gut can reduce food intake, animal studies have suggested that stimulation of the CB1 receptor in brain could induce an increase in food intake and body weight gain. It has been hypothesized that blocking the CB1 receptor would prevent weight gain, and drugs that antagonize CB1 (e.g. rimonabant) have been developed to target food intake and obesity. Anandamide and OEA, as mentioned above, have antiobesity effects when given orally or peripherally. It is conceivable that other endocannabinoids such as 2-acylglycerol (2-AG), the levels of which are enhanced in obesity, might contribute CB1-mediated increase in food intake [56,57]. FAAH inhibition would lead to higher levels of OEA and PEA (CB1 inactive) but not 2-AG. Although initial studies found some correlation between FAAH mutations and obesity, later studies have contradicted this [58–60]. FAAH-null mice do not show increased body weight. Thus, it seems unlikely that FAAs in brain contribute to increased food intake.

Cardiovascular effects of FAAs

FAAs, in particular anandamide, have been known to induce vasodilatation, modulate regional blood flow and arterial blood pressure, and reduce heart rate [61–65]. Both TRPV1 and CB1 might contribute to this activity. Anandamide is present in kidney endothelial and mesangial cells [66]. Anandamide at low concentrations stimulates CB1-receptor-mediated NO release from endothelial cells and produces a NO-mediated inhibition of KCl-stimulated norepinephrine release from sympathetic nerves. Thus, anandamide signaling in the kidney exerts significant vasorelaxant and neuromodulatory effects [66].

Endogenous FAAs have also been suggested to modulate heart stress and infarct size after injury by agents such as LPS [67,68]. The role of endogenous FAAs in cardiac protection was evaluated by Hajrasouliha *et al.* [69] in an ischemia/reperfusion injury model. In this model, remote preconditioning exerted potent cardioprotection (i.e. reductions in infarct size, as well as arrhythmias). CB2 receptor antagonist (but not CB1 antagonist) pretreatment abolished the protective effects of remote preconditioning on infarct size and arrhythmias, suggesting that endogenous cannabinoids, through acting on cannabinoid CB2 receptors, are involved in the cardioprotective phenomenon. Whereas CB2 receptors are present in cardiomyocytes, CB1 receptors are present in endothelial cells. Thus, FAA-mediated CB1 agonism might exert their effect through production of NO. By contrast, CB2 receptors present on cardiomyocytes exert a cardioprotective effect independent of NO.

In addition to NAEs, oleamide (*cis*-9,10-octadecenoamide) also shows protective vascular effects [70]. Oleamide induces vasodilation in rat small mesenteric artery. Vasodilation is mediated by endothelium-derived NO and through the activation of TRPV1 receptors. This activity also seems to require a pertussis toxin-sensitive GPCR, yet to be identified [70].

Modulation of endogenous FAA levels

On the basis of the discussion above, it is clear that FAAs possess many beneficial effects, not only in pain/CNS indications but also, potentially, in cardiometabolic diseases. FAA levels can be regulated through altered synthesis or degradation; the latter has received greater attention and has been the focus of therapeutic targeting. The FAAs like anandamide might be released from cells on demand by stimulus-dependent cleavage of membrane phospholipid precursors by the actions of NAT and PLD (Fig. 2). After release, anadamide might traverse within the plasma membrane and act on the receptors (e.g. CB1) of the same cell. Alternatively, anadamide might be released into the extracellular space and act on the receptors of neighboring cells. The activity of anadamide at its receptors is limited by cellular uptake, through a putative membrane transporter. The mechanism(s) governing the cellular transport of anadamide is controversial. Most reports indicate

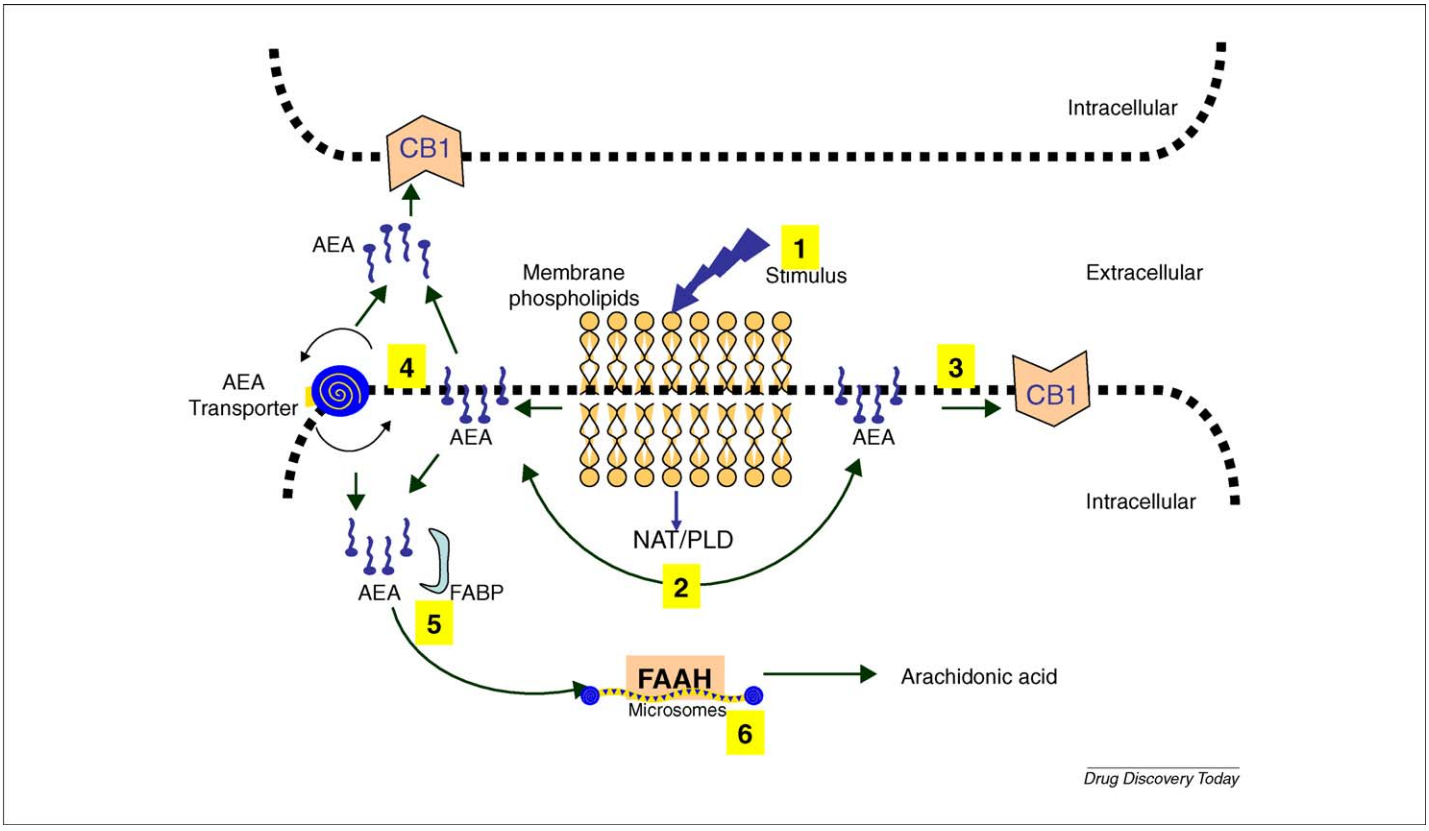


FIGURE 2

Pathways for anandamide (and other FAA) synthesis, transport and degradation. Numbers (yellow shade) refer to the sequence of events.

carrier-mediated uptake, whereas a few studies propose simple diffusion [71–73]. Experimentally, these two models have been distinguished by testing for saturability of anandamide uptake and its inhibition by selective compounds. A selective high-affinity anandamide membrane transporter (AMT) is believed to be important for transporting anandamide and other FAAs in both directions. After cellular uptake, anadamide and FAA are thought to be carried by specific fatty-acid-binding proteins (FABP5 or FABP7) to the microsomes, where they are hydrolyzed by FAAH [74]. It is believed that activation of CB1 receptor by anandamide releases NO that activates AMT for FAA uptake and internalization.

FAAH

Biology and function

FAAH is an intracellular enzyme that catalyzes the hydrolysis of endogenous FAA. FAAH is an integral membrane protein that belongs to a large family of enzymes that share a highly conserved 130 amino acid motif designated the 'amidase signature' sequence [75]. FAAH is well conserved in primary structure; the mouse and rat FAAHs share 91% amino acid identity, and the human FAAH shares >80% identity with the rat and mouse FAAHs. Human and rat FAAHs have similar localization and molecular size but distinguishable enzymological properties. FAAH is present in many tissues, including brain, intestine, liver, testes, uterus, kidney, ocular tissues, spleen and lung [75–80]. FAAH is absent from skeletal muscle and heart. Within the brain, FAAH expression varies from region to region, with the highest activities found in the globus pallidus and hippocampus and the lowest found in the medulla. In the brain, FAAH is often expressed in the same cells in which cannabinoid receptor CB1 is expressed. In the immune system, both lymphocytes and macrophages express FAAH, and the expression is either low or not found in platelets and polymorphonuclear leukocytes. FAAH is a membrane-bound enzyme and its association has been demonstrated with microsomal, mitochondrial and plasma membrane fractions.

Genetic FAAH deletions: pain and other therapeutic implications

FAAH inactivation by either genetic deletion or pharmacologic inhibition leads to highly elevated levels of endogenous FAAs. The half-life of anandamide in rat plasma and brain is believed to be less than 5 min [75,81]. However, when FAAH enzyme is inactivated or knocked out, the levels and half-life of anandamide and other FAAs increase. Tissue extracts from FAAH-null mouse showed 50- to 100-fold reduction in hydrolysis of anandamide and other FAAs, suggesting FAAH is the primary enzyme responsible for FAA degradation. The endogenous levels of anandamide and PEA are elevated more than tenfold in FAAH-null mouse brain [75,81–83]. Consistent with the different beneficial effects of FAAs described above, FAAH knock out results in analgesic, anxiolytic and antidepressant phenotypes [74,84–86]. FAAH-null mouse exhibited reduced inflammation in carrageenan-induced paw edema and dinitrobenzene sulfonic acid (DNBS)-induced intestinal colitis models [85,87]. In addition, age-related cardiac dysfunction and inflammatory gene expression were attenuated in FAAH-deficient mice [88]. The aging-associated systolic and diastolic dysfunction was less pronounced in aging FAAH-null mice than in wild-type mice. Furthermore, the aging-associated increased myocardial gene expression of inflammatory cytokines (TNFα) and degradative

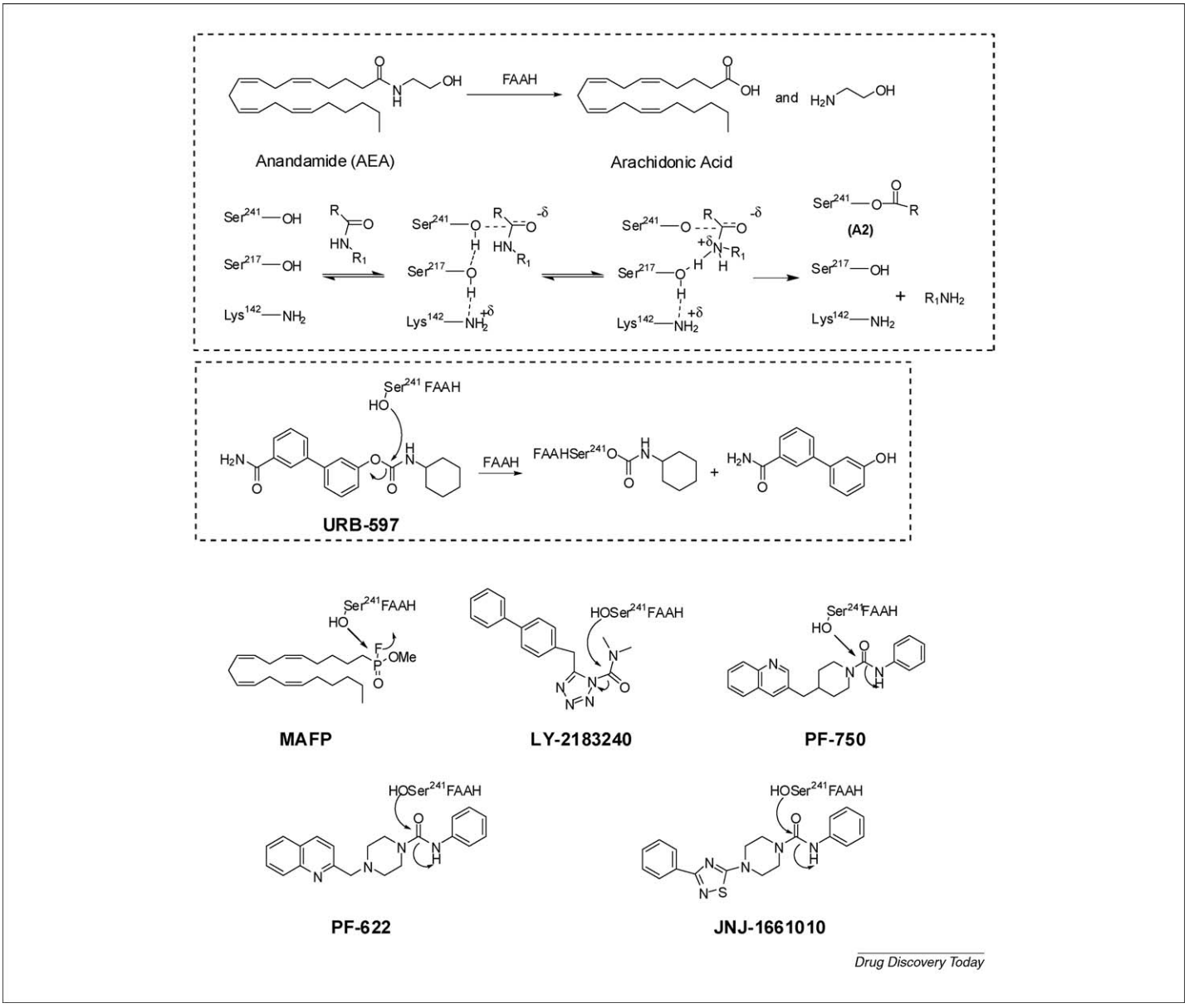


FIGURE 3

Irreversible inhibitors and mechanism of FAAH inactivation.

enzymes were also significantly decreased. These observations suggest that reduction or lack of FAAH results in hypoalgesia, reduced inflammation and improved cardiac function.

FAAH: structure and inactivation mechanism

The mammalian FAAH has three domains: an N-terminus transmembrane domain that dictates protein oligomerization, a serine–glycine-rich domain that contains amidase signature sequence and active site that contains a proline-rich domain, homologous to class II SH3-binding domain [74]. The architecture of FAAH gives useful insights on mechanism for substrate binding and product release. The structural organization of FAAH gives it simultaneous access to both the membrane and the cytoplasmic compartments. It has been hypothesized that the substrate enters FAAH through the membrane and traverses to the active site [74]. After hydrolysis, the hydrophobic fatty acid and the polar ethanolamine exit through the membrane access and the cytosolic access channels, respectively. It is believed that the cytosolic channel should also enable a water molecule to enter and hydrolyze the FFA–FAAH intermediate **A2** (Fig. 3) [74,89].

The X-ray crystal structure of rat enzyme (rFAAH) with an irreversible but nonselective inhibitor, methylarachidonyl fluorophosphonate (MAFP), has been published [90,91]. The enzyme uses a serine–serine–lysine (Ser241-Ser217-Lys142) catalytic triad site to hydrolyze the substrate. On the basis of the structural organization of the active-site triad, a concerted mechanism that involves nucleophilic activation of Ser241 by participation of Lys142 and bridging Ser217 has been proposed, as shown in Fig. 3 [91]. The lysine Lys142 acts both as a base in activation of Ser241 nucleophile and as an acid in protonation of substrate leaving group. The activation role played by Ser217 and Lys142 contributes to rapid hydrolysis of otherwise less reactive amides. FAAH seems to hydrolyze amides and esters at equivalent rates. More recently, a 'humanized' rat FAAH (h/r FAAH)

containing a human active site within rat enzyme has been engineered and cocrystallized with an irreversible inhibitor, PF-750 [92]. The aniline portion of PF-750 was not seen in the X-ray crystal structure of ligand–enzyme complex and gave useful insights on mechanism of covalent binding.

A second membrane-associated enzyme with a serine–serine–lysine triad has been identified recently in human cell lines and termed 'FAAH-2' [93]. FAAH-2 shows 20% homology with FAAH-1 and has distinct tissue distribution and substrate selectivity versus FAAH-1. FAAH-2 is present in primates but not in rodents. The role of FAAH-2 is not clear.

FAAH inhibitors

During the past few years, diverse classes of FAAH inhibitors have emerged, and these have been detailed in excellent reviews [94–98]. The segments below will give brief account of their evolution, salient features and scope.

Substrate mimetics

The initial medicinal chemistry approach on FAAH inhibitors relied on probing variants of endogenous FAA substrate. Early attempts on substrate mimetics, focusing on modification of hydrolytic amide site, yielded both reversible and irreversible inhibitors of FAAH. These molecules generally lacked selectivity and were also substrates for related hydrolases. For example, arachidonoyldiazomethylketone is a mixed inhibitor of FAAH and 5-lipoxygenase [99], and MAFP is a potent irreversible inhibitor of FAAH and cytosolic PLA2 [100,101]. Similarly, arachidonyl serotonin is a mixed inhibitor of FAAH (IC_{50} = 1.0–10 μM) and TRPV1 (IC_{50} = 37–40 nm) [102].

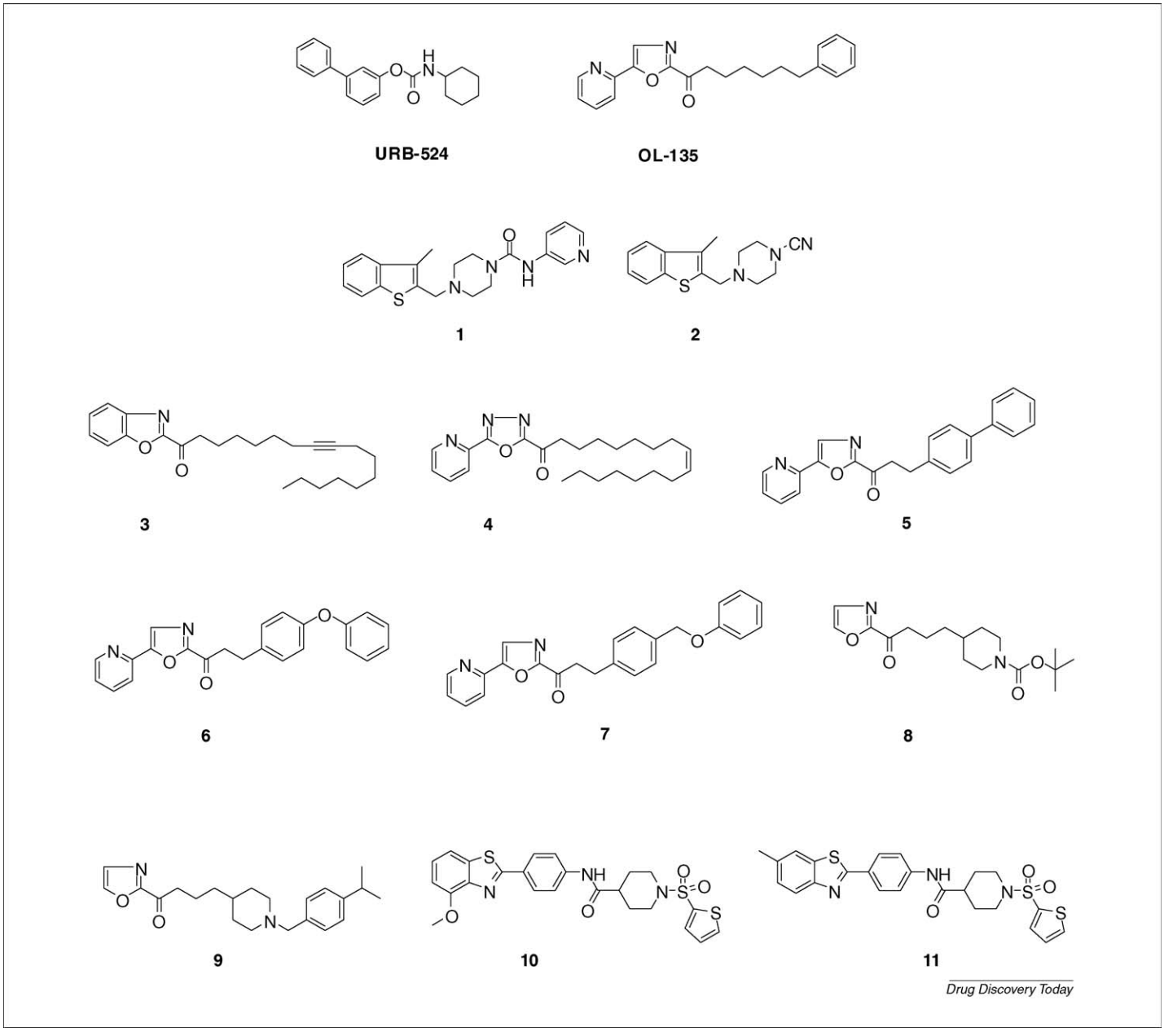


FIGURE 4

FAAH inhibitors.

Irreversible Inhibitors

Carbamate derivatives

As discussed above, FAAH is also blocked by inhibitors of other serine hydrolases. These are nonselective inhibitors that emerged as useful tools for exploratory research in many laboratories. In particular, the work done around the 'carbamate' class of serine hydrolase inhibitors, such as analogs of anticholinesterase agent carbaryl proved rewarding and led to identification of novel FAAH inhibitors [103–105]. The probing of structure–activity relationships suggested that biaryl-3-yl variations yielded a bent conformation and potent inhibitor, URB-524 (Fig. 4). In the optimization phase, introduction of polar groups such as carbamoyl-, hydroxyl- and hydroxymethyl- at the 3′ position gave very potent inhibitors, such as URB-597. URB-597 has been one of the most studied FAAH inhibitors. URB-597 inhibited FAAH with an IC_{50} = 4 nM in brain membranes and with an IC_{50} = 0.5 nM in intact neurons. It has been used as a tool to understand the mechanism of enzyme inactivation and to validate therapeutic potential of FAAH inhibition.

Biochemical evidence shows that the 'carbamate' class of inhibitors (URB-597) binds to the FAAH enzyme irreversibly at the active site through a nucleophilic attack by Ser241 (Fig. 3). Mass spectral evidence supports that URB-597 inhibits the enzyme by a covalent carbamoylation of its serine nucleophile. It also shows that biaryl substituents would reside in the cytoplasmic access channel rather than the acyl-chain binding channel to mimic the arachidonoyl moiety [105]. These insights have helped to generate more diverse and potent inhibitors of FAAH enzyme. Sanofi-Aventis has reported several diverse carbamate-containing FAAH inhibitors. The most advanced of these, SSR411298, is in a Phase II clinical trial for depression [106]. BMS have reported biaryl imidazole-derived carbamate inhibitors (IC_{50} < 10 nM) and oxime carbamoyl derivatives (IC_{50} ≤10 nM) as FAAH inhibitors [107,108].

Urea derivatives

Biaryl heterocyclic urea derivatives have been studied at Lilly [169]. These inhibitors bind to the enzyme irreversibly by carbamoylation of Ser241. The representative compound, LY-2183240, from the series inactivated enzyme with an IC_{50} = 12.4 nM and was effective under *in vivo* conditions [110]. These compounds seem to be less selective than typical carbamate and were shown to block other brain serine hydrolases under *in vivo* conditions. More recently, Di Marzo *et al.* [111] have continued to synthesize carbamoyl tetrazoles and examine their potential as FAAH inhibitors.

Johnson and Johnson has reported a series of thiadiazolopiperazinyl ureas as inhibitors of FAAH and discussed structure–activity relationship within the series [112]. JNJ-1661010 (Fig. 3) inhibits FAAH through acylation of Ser241. The compound was found to be more selective than URB-597 and reversible inhibitor OL-135 (Fig. 4) and did not cause liver esterase inhibition. The compound also showed good brain penetration and inhibited brain FAAH activity for over 24 hours with an approximate 1.4-fold increase in brain anadamide.

Pfizer has reported urea containing piperazine and piperidine-derived (PF-622 and PF-750) (Fig. 3) FAAH inhibitors with low nanomolar potency. In competitive proteome screening assays, the compound PF-750 seems to be highly FAAH selective and exhibited much superior selectivity against other serine hydrolases or carboxyesterases [92,113,114]. Interestingly, selectivity versus carboxyesterases seems to diminish in going from urea-1 to *N*-cyanamides-2 [114]. The compounds from the series are active under *in vivo* conditions in inflammation and OA-like pain models.

Reversible inhibitors

Among reversible inhibitors, compounds belonging to azetidinones (β-lactams) [115], imidazolidine-2,4-dione [116,117] and, in particular, keto-heterocycles series have been investigated in detail.

α*-Keto-heterocycles*

Since the pioneering work of Edwards *et al.* [109], compounds containing an electrophilic ketone and a structural template to capture active-site interactions have been used effectively to design inhibitors of diverse serine and cysteine proteases. Boger *et al.* have done extensive work in the area and used knowledge of the active-site pocket, synthetic ingenuity and structural–activity relationship to generate highly potent and selective inhibitors of FAAH [118–121].

In early work, Boger *et al.* combined an unsaturated long aliphatic acyl chain with α-oxazolo-heterocycles to give compounds, **3** and **4** (Fig. 4), with K_i values in subnanomolar range for FAAH inhibition. These compounds were also found to have good selectivity versus other serine hydrolases. Further work on side-chain modifications led to the identification of OL-135, which has been evaluated extensively by *in vitro* and *in vivo* models [122–124].

Extensive work on optimization of OL-135 series has been reported by Boger. Using a combination of computational and directed structure–activity relationship studies on the keto-heterocycles and hydrophobic connector chain, very potent and selective FAAH inhibitors, **5**–**7** (Fig. 4), have been identified [119,121].

Johnson and Johnson have worked on variations of phenhexyl chain of OL-135 with piperidine-derived chain modifications (compounds **8** and **9**, IC_{50} = 2–4 nM; Fig. 4). Compound **9** was found to be active in animal model of neuropathic pain [125].

Abbott recently reported benzothiazole analogs as FAAH inhibitors [126]. This novel series of compounds **10** and **11** (Fig. 4) were identified with respective activities, IC_{50} = 1.7 nM and IC_{50} = 18 nM), by optimization of hits from high-throughput screens.

Therapeutic potential

The FAAH inhibitors have been studied in numerous *in vivo* models to explore and validate therapeutic potential of FAAH inhibition [94–98,127]. Compounds URB-597 and OL-135 have been studied extensively and have the most data. The application of these compounds in the treatment of various pathologies (e.g. pain indications, inflammation-related disorders, neurological disorders or other indications) is summarized in Table 3. Both URB-597 and OL-135 are highly effective in different pain models, including inflammatory and neuropathic models. The effects of these inhibitors are diminished when coadministered with CB1 antagonists, suggesting that the CB1 receptor is a major contributor to the endogenous FAAH-mediated analgesic effect. However, unlike CB1 agonists, FAAH inhibitors do not affect motor

TABLE 3

Therapeutic applications of FAAH inhibitors in pain, inflammation, metabolic and cardiovascular indications

Indication	Summary of findings	Receptors	Refs
Pain			
Analgesia/nociception	Effective in thermal nociception and phenyl-*p*-quinone pain models	CB1/TRPV1	[110,144–148]
Neuropathic pain	Effective in spinal nerve ligation and chronic constriction injury models of neuropathic pain	CB1/CB2	[115,135–137,140, 145,147,149,150]
Inflammatory pain	Effective in formalin and carrageenan-induced inflammatory pain	CB1/CB2/PPARα	[115,134,143,147, 148,151–153]
Inflammation			
Cholestasis	Improved tail-flick latency in cholestatic rats		[154]
Inflammation/edema	Inhibits LPS-induced Cox2 and iNOS expression and decrease PGE2 levels. Effective in carrageenan-induced paw edema	CB2	[138,155]
Inflammatory bowel disease	Effective in trinitrobenzene-sulfonic acid induced experimental colitis	CB1/CB2	[139,156]
CNS			
Anxiety/depression	Anxiolytic-like antidepressant-like effects in mouse and hamster models without cannabinoid-like effects (e.g. catalepsy, hypothermia, hyperphagia)	CB1	[86,110,157–163]
Nausea/emesis	Prevented vomiting produced by cisplatin and nicotine, lithium induced nausea	CB1	[164–166]
Parkinson's disease (and dyskinesis)	Decreased all abnormal involuntary movements only if coadministered with the TRPV1 antagonist capsazepine	TRPV1	[167,168]
Pruritus	Inhibited mast-cell degranulator compound 48/80 evoked scratching responses	CB1	[169]
Tobacco dependence (nicotine addiction)	Prevented development of nicotine-induced conditioned place preference and acquisition of nicotine self-administration		[170]
CV and metabolic			
Hypertension	Decreased arterial pressure in spontaneous hypertensive to normotensive levels. Also decreased left ventricular systolic pressure, and total peripheral resistance index Lowered blood pressure in angiotensin-II-treated hypertensive rats but not in their normotensive controls	CB1	[142]
Diabetes/obesity	Prevents gastric emptying in mice fed on normal or high-fat diet	CB1	[51,139]
	Potentiates GLP-1 secretion from intestinal cells	GPR119	[141]

performance. FAAH inhibitors have shown very good antidepressant-like effects in the mouse models (the tail-suspension test and the rat forced-swim test). FAAH inhibition increases firing activity of serotonergic neurons in the dorsal raphe nucleus and noradrenergic neurons in the nucleus locus ceruleus. These activities are prevented by the CB1 antagonist rimonabant and are accompanied by increased brain anandamide levels. Unlike direct CB1 agonists, URB-597 does not evoke classical cannabinoid-like effects (e.g. catalepsy, hypothermia and hyperphagia), does not cause place preference and does not produce generalization to the discriminative effects of the active ingredient of cannabis, Δ^9-tetrahydrocannabinol.

Although the potential of FAAs in metabolic and cardiovascular areas have been studied extensively, the potential of FAAH inhibitors in these indications is only beginning to be realized. FAAH inhibitors possess potent anti-inflammatory effects and, thus, could have therapeutic effects in diseases in which inflammation is the underlying cause. URB-597 inhibits LPS-induced Cox2 and iNOS expression and decreases PGE2 levels [128]. In animal models of colonic inflammation, CB1 agonists and FAAH inhibitor normalized croton-oil-induced hypermotility [129]. As discussed in the previous sections, FAAs can regulate intestinal motility and food intake. In animal models, gastric emptying can be evaluated by measuring the amount of phenol red recovered in the stomach after oral challenge. Anandamide and FAAH inhibitor arachidonoylserotonin reduced gastric emptying [48]. FAAH inhibitor also affected gastric emptying more efficaciously in mice fed on high-fat diet than in mice fed normal diet. This effect is only partly inhibited by CB1 antagonist rimonabant, suggesting other receptors contribute to the effects of FFAs. These effects are similar to those seen with incretin GLP-1, which limits gastric emptying and promotes weight loss [130].

Intestinal L-cells secrete GLP-1 in response to ingestion of nutrients, especially long-chain fatty acids. Long-chain fatty acids are converted to FAAs (e.g. OEA), which can bind and activate GPR119 and promote GLP-1 secretion. GLP-1 secretion from intestinal cells can be enhanced by the FAAH inhibitor URB-597 [131]. Endogenous OEA levels are enhanced during feeding; thus, FAAH inhibitors might show better effects when given before feeding. These effects need to be explored in animal models of obesity/diabetes.

The therapeutic potential of FAAH inhibitors has also been explored in models of vascular dysfunction. In rat isolated small mesenteric arteries, FAAs and URB-597 induced relaxation [28]. Treatment with URB-597 potentiated the depressor and mesenteric vasodilator responses to anandamide. The effects of URB-597 were tested in rat model of spontaneous hypertension (SH) [132]. SH rats have significantly elevated blood pressure compared with age-matched control rats. In myocardium, FAAH expression was unexpectedly increased more than twofold and anandamide levels decreased correspondingly in SH rats compared with controls. Treatment with URB-597 (10 mg/kg IV) caused a greater than twofold increase in anandamide levels in SH rats than in control rats. URB-597 also caused an increase in plasma anandamide levels (1.78 ± 0.20 versus 2.44 ± 0.20 pmol/mL, or 1.3-fold). Treatment of normal rats with URB-597 had no detectable hemodynamic

effects, whereas in SH rats, URB-597 decreased arterial pressure to normotensive levels for >30 min. URB-597 also decreased left ventricular systolic pressure and total peripheral resistance index. A URB-597-induced decrease in cardiac contractility in SH rats was also indicated by the change in pressure/volume relationship. URB-597 similarly lowered blood pressure in angiotensin-II-treated hypertensive rats but not in their normotensive controls [133]. The effects of URB-597 in the hypertensive animals could be prevented by CB1 antagonists, suggesting the contribution of CB1 receptor to the beneficial effects of endogenous FAAs.

Concluding remarks

Endogenous FAAs can interact with multiple pain receptors and have demonstrated excellent therapeutic utility in pain. Although the beneficial effects of exogenously administered FAA have also been extensively studied in metabolic and cardiovascular diseases, the role of endogenous FAAs is only beginning to be realized. Because FAAH is the major enzyme responsible for endogenous FAA inactivation, FAAH inhibition is an attractive therapeutic strategy to realize the potential of endogenous FAAs in pain and nonpain settings. During the past few years, there has been very good progress in understanding active-site interactions and mechanism of FAAH enzyme inactivation. Diverse classes of molecules, which have excellent inhibitory potency and significantly improved selectivity profiles, have been discovered. FAAH inhibitors have been validated preclinically in pain, anxiety, depression, neurological disorders, metabolic and hypertension models. One of these molecules (SA-41129) are entering into the clinic. FAAH inhibitors would not only effectively address the limitations of the current pain medications (i.e. GI and cardiovascular side-effects of NSAIDs and abuse potential of opioids) but also be likely to have independent therapeutic potential in metabolic and cardiovascular diseases. Although FAAH inhibition seems to lack the CNS side-effects of cannabinoids, FAAH-mediated anandamide regulation appears to play a key part in embryo implantation, which could be clinically relevant for fertility regulation in women [134]. It remains to be determined whether FAAH inhibitors would have adverse effects in pregnancy that might limit their use in a select population.

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