Focus on the Three Key Enzymes Hydrolysing Endocannabinoids as New Drug Targets

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Abstract: The family of endocannabinoids (i.e., the endogenous agonists of cannabinoid receptors) contains several polyunsaturated fatty acid amides such as anandamide (AEA) and oleamide but also esters such as 2-arachidonoylglycerol (2-AG). These compounds are the subject of growing interest in pharmacology for their multiple therapeutic potentials. Unfortunately, they are rapidly inactivated by enzymatic hydrolysis, which prevents their effective medical use. Inhibitors of endocannabinoid degradation seem to be necessary tools for the development of endocannabinoid therapeutics. But hitting this target is inconceivable without good knowledge of the enzymes. Fatty acid amide hydrolase (FAAH) is the oldest and the best characterised enzyme involved in the degradation of endocannabinoids. Cloning, distribution in the body and crystal structure of FAAH have been described. A large number of FAAH inhibitors have also been synthesised and tested. For a long time, FAAH was considered as the only key enzyme hydrolysing endocannabinoids. But recent findings indicate that at least two other enzymes have critical role in the endocannabinoids degradation. Monoglyceride lipase participates in 2-AG degradation and some data indicate that it is the primary mechanism for 2-AG inactivation in intact neurons. *N*-palmitoylethanolamine-selective acid amidase (NPAA) is a second fatty acid amide hydrolase more active with *N*-palmitoylethanolamine, an anti-inflammatory substance. The purpose of this review is to collect and compare the catalytic properties of these 3 key enzymes hydrolysing endocannabinoids.

INTRODUCTION

The biological effects of marijuana and Δ^9-tetrahydrocannabinol (THC), its major psychoactive component, are mediated by two cannabinoid receptors christened CB_1 and CB_2. Both receptors are seven transmembrane domain receptors coupled through $G_{i/o}$ proteins. CB_1 receptor has been cloned in 1990 [1] and it is abundantly found in the brain and the central nervous system [2, 3]. CB_2 cloning was succeeded in 1993 [4] and this second receptor is associated with the immune system and some periphery organs like the spleen [5]. But the physiological role of these receptors does not restrict itself to the binding of the constituents of a plant. The quest for endogenous ligands for the cannabinoid receptors leads to the discovery of a family of polyunsaturated compounds derived from arachidonic acid (Fig. **1**).

Anandamide (arachidonoylethanolamide, AEA) was the first "endocannabinoid" (i.e., endogenous agonist of cannabinoid receptors) isolated from brain lipid extracts by William Devane [6]. AEA shares properties with Δ^9-THC [7] and exhibits partial agonist activity on cannabinoid receptors CB_1 and CB_2 *in vitro* [8, 9]. AEA induces antinociception in the formalin-evoked pain [10, 11], in the tail-flick model applied to rhesus monkeys [12], in non-arthritic and arthritic rats [13], in carrageenan model applied to mice [14] and rats [15], and after turpentine injection into the rat bladder [16] (for reviews about antinociceptive effects of anandamide, see Pertwee [17] and Rice [18]). Tremendous research on anandamide was also dedicated to its effect on cell proliferation. Anandamide induces apoptosis in human neuroblastoma CHP100, human lymphoma U937 [19] , PC-12 cells [20], human prostatic cancer cells [21], endothelial cells [22], non-differentiated CaCo-2 cells [23], breast cancer cells [24] and C6 glioma cells. However, in this last case, it is not clear whether the effect of AEA is really due to the compound itself or to its metabolite arachidonic acid [25] (for reviews about antiproliferative effect of anandamide, see Maccarrone [26] and Parolaro [27]). AEA also plays a role in reproduction [28, 29], memory processes [30-33], attenuation of cholera toxin-induced fluid accumulation [34], modulation of anxiety [35], increasing of cytochrome P450 content and activity in rat [36], modulation of epilepsy [37] and modulation of feeding [38, 39].

2-arachidonoyl glycerol (2-AG) (Fig**. 1**) was the second endocannabinoid discovered some years after anandamide. 2-AG was isolated from canine gut by Mechoulam *et al*. [40] and from brain by Sugiura *et al.* in 1995 [41]. In 1997, Stella *et al.* reported that 2-AG is present in brain in amounts 170 greater than anandamide [42]. Functional studies demonstrated later that, unlike AEA, 2-AG is a full agonist of the CB_2 receptor [43, 44] but also of CB_1 cannabinoid receptor [45]. Pharmacological properties of 2-AG include inhibition of long-term potentiation [42], inhibition of proliferation of breast and prostate cancer cells [46], induction of hypotension [47, 48], contractile action [49], neuroprotection after brain injury [50], attenuation of naloxone-precipitated withdrawal signs in morphine-dependent mice [51], stimulation of nitric oxide release [52] and stimulation of appetite of mice pups [53].

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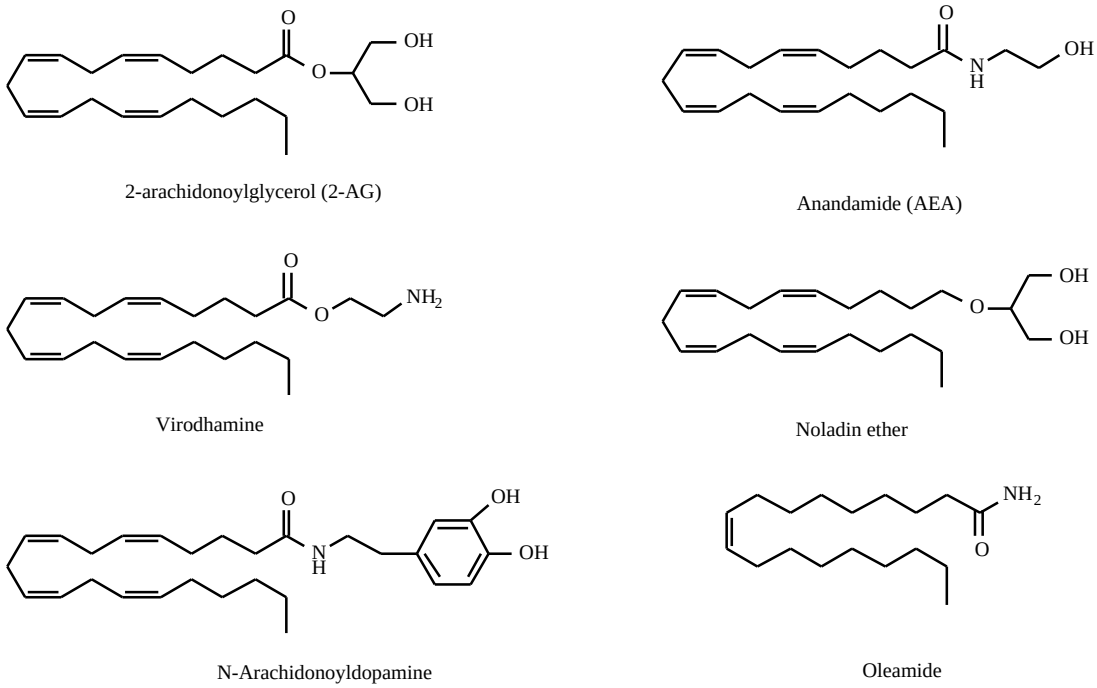


Fig. (1). structures of the six putative endocannabinoids

The family of endocannabinoids grew larger during the last years with the discovery of noladin ether, virodhamine, arachidonoyldopamine (NADA) and oleamide. Noladin ether was isolated from porcine brain in 2001, it only binds to the CB_1 receptor [54] and it prevents, as anandamide does, neurotoxicity of the human amyloid-β peptide [55]. Nevertheless, the endogenous existence of noladin ether was recently called into question [56]. One year later, virodhamine (*O*-arachidonoyl ethanolamine) (Fig. **1**) was extracted from rat brain. It acts as a partial agonist at CB_1 receptor, and full agonist at CB_2 receptor [57]. In 2000, Bisogno *et al*. found that NADA (Fig. **1**) acts as a selective CB_1 agonist and it inhibits proliferation of human breast cancer cells [58]. Evidence of the endogenous existence of NADA was succeeded two years later by its isolation from rat brains and bovine dorsal root ganglions [59], which assign it the membership of the "endocannabinoids family". Finally, it has been shown recently that oleamide [60] is an endogenous full CB_1 receptor agonist [61] which induces hypnotic action blocked by a CB_1 receptor antagonist [62].

In addition to these endocannabinoids, other endogenous fatty acid amides ligands have been identified. They are characterised by cannabimimetic properties *in vitro* and *in vivo;* however, they are devoid of affinity for the cannabinoid receptors. It is likely that these ligands interfere with the degradation of endocannabinoids and consecutively enhance their levels and strengthen their biological actions mediated by CB_1 and CB_2 receptors. Such a mechanism has been described as an "entourage effect" in the literature [63]. Stearoylethanolamide (Fig. **2**) has the same effects as AEA on catalepsy, motility, analgesia and body temperature but it has no affinity for cannabinoid receptors [64]. Palmitoylethanolamide (PEA) is a scheming compound, which was first attributed as an endogenous CB_2 ligand. Facci reported that PEA was able to inhibit binding of [^{3}H]-WIN 55, 212-2 to RBL-2H3 basophilic leukaemia cell membranes [65]. However, other groups, including ours, were unable to detect an interaction between PEA and CB_2 receptor in other tissues [66, 67]. It is now generally assumed that PEA does not bind to CB_1 and CB_2 cannabinoid receptors. PEA is an anti-inflammatory compound, which can, after orally administration, reduce degranulation of mast cells produced by injection of substance P into the ear pinna [68]. PEA induces antinociception in formalin-evoked pain, which is reversed

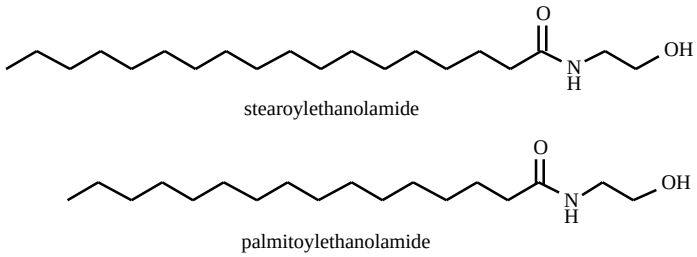


Fig. (2). Structures of stearoylethanolamide and palmitoylethanol-amide.

by the CB_2 antagonist SR 144528 [11], [69]. PEA is also effective after injection of turpentine into the urinary bladder [10] and it acts synergistically with AEA to inhibit formalin-evoked and kaolin-evoked pain behaviour [69] (for reviews on the antinociceptive effect of PEA, see Lambert [70] and Walker [71]). PEA is a potent anticonvulsant, devoid of neurologic impairment, in maximal electroshock seizure test [72]. PEA also has antiproliferative properties on human breast cancer cells, by a mechanism, which involves a down-regulation of the enzyme responsible for AEA degradation [73].

After the discovery of the family of endocannabinoid ligands, searchers questioned the existence of a mechanism able to terminate their signalling activity. Ten years ago, Deutsch and Chin reported the identification of an "anand-amide amidase" [74] in neuroblastoma and glioma cells, which was later referred to as "anandamide amidohydrolase" in rat and porcine brains by other research groups [75, 75]. In 1996, the purification and characterisation of an "oleamide hydrolase" by Cravatt *et al*. put an end to the plurality of denominations. The same enzyme was able of hydrolysing both anandamide and oleamide, and it was renamed "fatty acid amide hydrolase" (FAAH) [77]. For a long time, FAAH was considered as the only key enzyme hydrolysing endocannabinoids. But recent findings indicate that at least two other enzymes have a critical role in the endocanna-binoids degradation. During the ten years elapsed after the discovery of "anandamide amidase" by Deutsch and Chin, huge progresses have been achieved in its characterisation. Cloning, distribution in the body and crystal structure of FAAH has now been described. However, the same time has allowed the identification of two other enzymes, namely monoglyceride lipase [78], which participates in 2-AG degradation, and *N*-palmitoylethanolamine-selective acid amidase (NPAA) [79], for which PEA is the most active substrate.

If the synthesis of inhibitors of endocannabinoid degradation is a necessary tool for the development of endocannabinoid therapeutics, future researches will be indissociable of the entirety of the three enzymes, and will be focused on design of selective inhibitors of each of them. We propose along this review, to collect and compare the catalytic properties of these 3 key enzymes hydrolysing endocannabinoids.

FATTY ACID AMIDE HYDROLASE (FAAH)

Molecular Structure

FAAH is a membrane-bound enzyme, which belongs to the family of amidases enzymes. They are characterised by a highly conserved region rich in serine, glycine and alanine residues. This region, called "amidase signature" is common to more than 80 amidases enzymes and it corresponds to amino acids 215-257 in mammalian FAAH enzymes. FAAH from rat was first successfully cloned by Cravatt *et al.* in 1996. Human [80], mouse [80] and porcine [81] FAAH were subsequently cloned and have shown 73% identity at the overall amino acid level and 90% identity at the amidase signature. The FAAH proteins of these different species are all 579 amino acids in length, however their mRNAs differ in size (for more details on molecular structure of FAAH, see reviews Ueda and Yamamoto [82] and Ueda [83]). The 2.8 Å X-ray crystal structure of FAAH was reported by Bracey *et al*. [84] and constitutes a major progress in the under-standing of the three-dimensional structure of this enzyme. This study revealed that FAAH is a dimeric enzyme with a core composed of a serine-serine-lysine triad. FAAH possesses channels to establish direct access between the bilayer and its active site, to facilitate substrate binding and products release. Recently, the characterisation of an *Arabidopsis thaliana* FAAH homologue was reported by Shrestha *et al*. [85]. Its cDNA encodes a protein of 607 amino acids with 37% identity to rat within the amidase signature.

Distribution

In rat, FAAH is mainly distributed in the liver, small intestine, brain, testis, uterus, kidney, occular tissues and spleen, but not in skeletal muscle or heart [74, 75, 86]. In human, the distribution is different: FAAH was mainly detected in pancreas, brain, kidney, skeletal muscle [80], placenta [87] and less abundant in liver [80]. FAAH activity was also detected in mouse uterus [88], and its expression is regulated during pregnancy [89, 90]. FAAH activity was also detected in several ocular tissues including bovine retina [91], rat retina [92], porcine retina, optic nerve, iris and lacrimal glands [93]. Within the brain, activity of FAAH was the highest in globus pallidus, hippocampus, substantia nigra, cerebellum, cerebral cortex, cerebellum and the lowest in brainstem and medulla [94]. Immunohistochemical studies showed the preferentially localisation of FAAH in pyramidal cells of cerebral cortex, hippocampus, purkinje cells of cerebellar cortex and mitral cells of olfactory bulb [95]. Recently, Romero *et al*. performed an immunohistochemical analysis in hippocampus and enthorinal cortex sections from brains of Alzheimer's disease patients. They showed that FAAH is abundantly and selectively expressed in neuritic plaque associated astrocytes and microglia, and that its activity is elevated in the plaques and surrounding areas [96]. It is also significant to note that FAAH and CB_1 receptor have complementary localisation in the retina [92], the placenta [87] and the brain [97, 98], where FAAH is preferentially located in the large neurons postsynaptic to CB_1 [95, 97].

In cell lines, FAAH was detected in mouse N18TG2 neuroblastoma [74, 99], rat C6 glioma [74], J774 macro-phages [100], RBL-2H3 basophilic leukaemia cells [101], bronchioalveolar non-small cell lung carcinoma [74] and human neuroblastoma CHP199 [102]. However, attempts to detect FAAH failed in human epithelioid carcinoma (HeLa), human larynx epidermoid carcinoma (Hep2), human hepato-cellular carcinoma (HepG2) [74] and monkey COS-7 kidney fibroblast-like cells [103]. Human embryonic kidney cells (HEK) constitute a scheming case in term of FAAH expres-sion. If FAAH expression and activity were detected in human vanilloid receptor (VR_1) transfected HEK cells [104], Western blotting [105] and reverse transcriptase-polymerase chain reaction (RT-PCR) (Vandevoorde S, Delouvroy F and Lambert DM., unpublished result) failed to detect any expression of FAAH in non-transfected HEK cells.

Assays

Monitoring of the hydrolysis of anandamide to arachidonic acid and ethanolamine was achieved by using of labelled, either on the arachidonoyl moiety or on the ethanolamine moiety, anandamide. When [^{14}C]- or [^{3}H]-anandamide labelled on the arachidonoyl moiety was used, separation of the products of hydrolysis was achieved by thin-layer chromatography [74, 76, 77] or by high-performance liquid chromatography [106]. When [^{14}C]- or [^{3}H]-anandamide labelled on the ethanolamine moiety was used, the released ethanolamine was separated by open bed column chromatography [75], [99] or by methanol-chloroform extraction [107, 108]. Very recently, a novel high-throughput-compatible assay, based on the differential absorption of the substrate and the hydrolysis products to activated charcoal was reported [109].

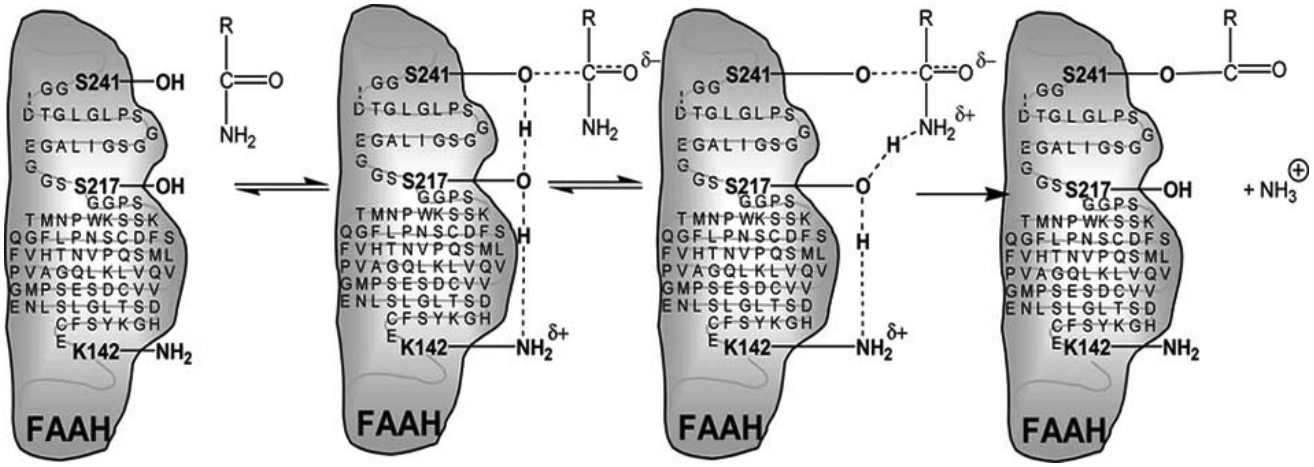
Non-radioactive assays were also developed. Separation and quantification of arachidonic acid product was achieved by gas chromatography [110] and high-performance liquid chromatography (HPLC) [111, 112]. HPLC was also used for the separation and detection of released ethanolamine moiety [113]. A fluorescence assay [114], and a calorimetric assay using fatty acid *p*-nitroanilides [115] were also reported.

Substrates

FAAH is capable of hydrolysing a wide variety of substrates. Substrate specificities are dependent of enzyme preparations (from rat liver, rat or porcine brain, rat, human and porcine recombinant, N18TG2 or RBL-2H3 cells - A comprehensive table of substrate specificities of FAAH with various enzyme preparations is available in a recent review of Deutsch [116]). As an example, PEA is rapidly metabolised by enzymes from rat liver [117] and mouse N18TG2 cells [98], but hardly by enzymes from rat [75] and porcine [76] brain. However, AEA was revealed as the most active substrate in all the studies. FAAH exhibits a high amidase activity against anandamide (C20:4), stearoylethanolamide (C18:0), palmitoylethanolamide (C16:0), myristoylethanolamide (C14:0), lauroylethanolamide (C12:0), oleoylethanolamide (C18:1) and oleamide. In addition to its amidase activity, FAAH exhibits esterase activity against 2-arachidonoylglycerol (C20:4) [112], methyl arachidonate and oleoyl methyl ester. Patricelli *et al.* reported that FAAH hydrolyses oleoyl methyl amide and its analogue oleoyl methyl ester with equivalent catalytic efficiencies. Mutation of a single lysine residue to alanine (K142A) abolished this property and produces a mutant FAAH enzyme hydrolysing esters more than 500-fold faster than amides [118].

Catalytic Mechanism

In 1999, two distinct groups investigated the catalytic features of FAAH through mutagenesis studies. They found that three serine residues (S217, S218 and S241) are required for FAAH activity [119, 120]. In 2000, Patricelli and Cravatt conducted a comprehensive study of mutagenesis of all potentially catalytic residues in FAAH, highly conserved in all amidase signature enzymes. They attributed some residues to primarily structural roles and they found that residues Lys-142, Ser-217, Ser-218, Ser-241 and Arg-243 are critical for amidase activity [121] . The 2.8 Å X-ray crystal structure of FAAH recently reported by Bracey *et al.* [84] confirmed that FAAH is a dimeric enzyme with a core composed of a serine-serine-lysine triad. Very recently, Mc Kinney proposed a catalytic mechanism through the formation of a tetrahedral intermediate. Uncharged Lys-142 initiates catalysis by accepting a proton from Ser-217, which in turn deprotonates Ser-241 nucleophile to facilitate attack on the substrate carbonyl (scheme **1**) [122].



Scheme 1. Proposed mechanism for the acylation step of amide hydrolysis by FAAH. (scheme adapted from the scheme 1 of Mc Kinney and Cravatt [122]).

Successive deprotonations from Ser-217 to Lys-142 and from Ser-241 to Ser-217 (A) allow the attack of the substrate carbonyl by Ser-217, which gives a proton to the nitrogen atom of the amide group of the substrate (B). Simulaneously, Lys-142 gives a proton to Ser-217. These protons transfers induce the return of Lys-142 and Ser-217 in their initial protonation states and result in the formation of an acyl enzyme intermediate (C).

Reversibility of Anandamide Hydrolysis

In 1994, two research groups reported the synthesis of anandamide by condensation of arachidonic acid and ethanolamine on brain membranes. They demonstrated that the synthesis of anandamide occurs through an ATP- CoA-independent process [123, 124]. Five years later, the equilibrium in the hydrolysis and synthesis of anandamide was demonstrated by a purified FAAH enzyme [125]. FAAH was then assumed to work not only as hydrolase, but also as anandamide synthase. However, it was also demonstrated that very high concentrations of ethanolamine and arachidonic acid are required for the synthase pathway by FAAH, and that this enzyme could not work as a synthase under physiological conditions. It is now generally assumed that the biosynthesis of anandamide occurs by a mechanism, which involves the formation of *N*-arachidonoyl-phosphatidylethanolamine catalysed by a calcium-dependent transacylase, followed by the hydrolysis by a phosphodiesterase of the D type [126]. Very recently, Okamoto *et al.* reported the cloning of a phospholipase D responsible of AEA biosynthesis [127].

Inhibitors

The multiple therapeutic potentials of endocannabinoids are well-established. Unfortunately, these compounds are rapidly inactivated by FAAH enzymatic hydrolysis, which prevents their effective medical use. Inhibitors of FAAH seem to be necessary tools for the development of endocannabinoid therapeutics: drugs that block inactivation of endocannabinoids might be beneficial in disease states in which endocannabinoid levels are down-regulated. Inactivation of FAAH was already shown to elicit pain and anxiety [35] without the side effects (hypomotility, hypothermia and catalepsy) accompanying activation of CB_1 receptors by exogenous cannabinoids like Δ^9-THC (for reviews of therapeutic potentials of endocannabinoids, see Piomelli [128]; Fowler [129] and Cravatt [130]). To preserve this lack of "cannabinoid side effects", the inhibitors must be devoid of affinity for the cannabinoid receptors, especially for the cannabinoid receptor 1, which is involved in most of the side effects of exogenous cannabinoids. Data on the inhibitory potential against FAAH are now indissociable of the data regarding affinity for the cannabinoid receptors. For this reason, we considered important and useful to mention the data on affinities for the cannabinoid receptor 1 for the inhibitors listed in the following tables. FAAH is characterised by an optimum alkaline pH ranging from 8.5 to 10 [107]. However, most of the inhibitory assays are performed at physiological pH. Because a modification of the pH assay can induce drastic changes in inhibitors potencies, we also considered useful to indicate the pH assay in parallel of the Ki and IC_{50} values reported.

IRREVERSIBLE INHIBITORS OF FAAH

When Deutsch and Chin [74] discovered FAAH in 1993, they also observed that phenylmethylsulfonyl fluoride (PMSF) was a potent inhibitor. This observation was confirmed by Desarnaud *et al.* [75] in 1995. However, this well-known serine protease inhibitor was not selective for FAAH and a growing interest was rapidly shown in the design of more selective inhibitors. To reach this goal, searchers took their inspiration from the chemical structures of the substrates of FAAH, and developed derivatives of anandamide. This method allowed the discovery of methyl arachidonoyl fluorophosphonate (MAFP) [131, 132], diazomethylarachidonoyl ketone (DAK) [133], laurylsulfonyl fluoride [134], stearylsulfonyl fluoride [134], methyldodecyl fluorophosphonate [135] and very recently arachidonyl sulfonyl fluoride [136]. All these compounds are potent inhibitors of FAAH, however, they also have remarkable affinity for the CB_1 receptor.

In 1998, Patricelli et *al.* identified 2-octyl γ-bromoacetate, an endogenous compound originally isolated from human cerebrospinal fluid, as a potent irreversible inhibitor of FAAH [137]. Interestingly, at pH 7.0, this compound was proved to be significantly more potent than oleoyl trifluoromethyl ketone (a potent transition state inhibitor of FAAH that we will discuss in the next part). In 1997, Beltramo reported that the bromoenol lactone, *(E)*-6-(bromomethylene) tetrahydro-3-(1-napthtalenyl)-*2H*-pyrano-2-one (BTNP) is a non-competitive and irreversible inhibitor of FAAH [138]. However, the affinities of these compounds for the cannabinoid receptors were not investigated.

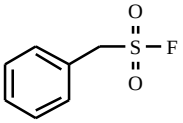
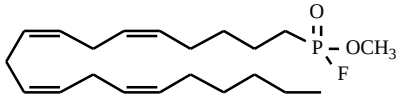
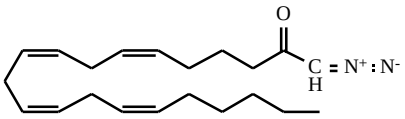
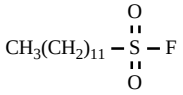
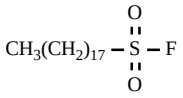
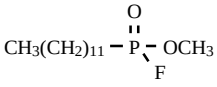
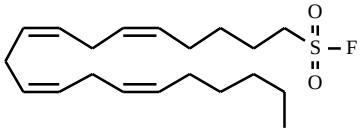
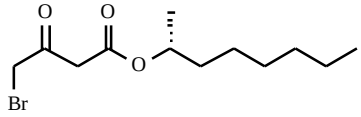
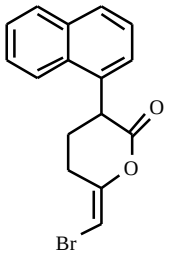
Very recently, Kathuria *et al.* described a new class of FAAH inhibitors. URB597 (carbamic acid, cyclohexyl-3'-(aminocarbonyl) [1, 1'-biphenyl]-3-yl ester), the most potent, does not exhibit affinity for the cannabinoid receptors. Thus, at doses that inhibit FAAH and substantially raised brain anandamide, but not 2-AG, levels, this compound does not evoke catalepsy and hypothermy. However, URB597 exhibits anxiolytic-like actions, which are prevented by CB_1 receptor antagonist SR 141716A [35]. Alkylcarbamic acid aryl esters constitute a new class of inhibitors a FAAH described by Tarzia in 2003. The most potent members of this family are *N*-cyclohexylcarbamic acid biphenyl-3-yl ester and *N*-butylcarbamic acid –4-(phenylmethoxy) phenyl ester, which are devoid of affinity for cannabinoid receptors (Table **1**) [139].

OTHER INHIBITORS OF FAAH

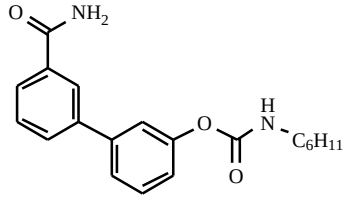
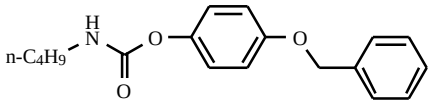
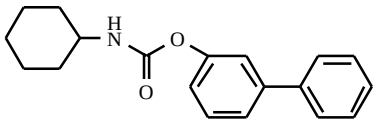
Modification of the chemical structure of substrates of FAAH does not only lead to the formation of irreversible inhibitors. In 1994, Koutek reported the synthesis of arachidonoyl trifluoromethyl ketone (ATFMK), a transition-state inhibitor of FAAH. ATFMK was able to displace the [^{3}H]-CP 55, 940 binding to CB_1 with a Ki of 0.65 μM [140]. One year later, Patterson described the synthesis and evaluation of the trifluoromethyl ketone derivative of oleamide [141].

An alternative to transition state inhibitors is to use derivatives of the endogenous lipid palmitoylethanolamide (PEA), since this compound is able to prevent the FAAH-catalysed metabolism of anandamide (AEA) [142] and to inhibit the expression of FAAH [143]. Palmitoylisopropylamide exhibits good inhibitory potential against FAAH not only in presence of rat brain homogenates but also in intact C6 cells, in which it produces 87% inhibition of [^{3}H]-ethanolamine production from [^{3}H]-AEA, at 30 μM [143]. *N*-(1-Oxohexadecyl)glycine methyl ester and *N*-(2-acetoxyacetyl)pentadecylamine, very recently described, inhibit FAAH with IC_{50} values of 10 and 8.3 μM, respectively

Table 1. Irreversible Inhibitors of FAAH

Chemical structure	Name	FAAH inhibition		Affinity for CB_1 receptor		References
		Ki / IC_{50} (assay pH)	**Radioligand used, source of FAAH**	**Ki / IC_{50}**	**Radioligand used, source of CB1 receptor**	
	Phenylmethylsulfonyl fluoride (PMSF)	IC_{50} 25 µM (pH 7.5)	[^{3}H]-AEA rat brain homogenates	-	-	Desarnaud [75]
		IC_{50} 900 nM (pH 7.6)	[^{14}C]-AEA rat brain homogenates	IC_{50}> 10 µM[134]	[^{3}H]-CP 55940 rat brain membranes	Deutsch [131] Deutsch [134]
	Methyl arachidonoyl fluorophosphonate (MAFP)	IC_{50} 3 nM (pH 7.6)	[^{14}C]-AEA rat brain homogenates [131] $N_{18}TG_2$ cells [132]	IC_{50} 20 nM	[^{3}H]-CP 55940 rat brain membranes	Deutsch [131]
		IC_{50} 1 nM (pH 9.0)	[^{14}C]-AEA RBL-1 cells [132]			De Petrocellis [132]
	Diazomethylarachidonoyl ketone (DAK)	IC_{50} 6µM (pH 9.0)	[^{14}C]-AEA porcine brain homogenates			De Petrocellis [132]
		IC_{50} 3 µM (pH 9.0)	[^{14}C]-AEA $N_{18}TG_2$ cells			
		IC_{50} 2 µM (pH 9.0)	[^{14}C]-AEA RBL-1 cells			
		IC_{50} 520 nM (pH 7.4)	[^{3}H]-AEA rat brain membranes	Ki 1,3 µM	[^{3}H]-CP 55940 rat brain membranes	Edgemond [133]
	Laurylsulfonyl fluoride	IC_{50} 3 nM (pH 7.6)	[^{14}C]-AEA rat brain homogenates	IC_{50} 18,4 nM	[^{3}H]-CP 55940 rat brain membranes	Deutsch [134]
	Stearylsulfonyl fluoride	IC_{50} 4 nM (pH 7.6)	[^{14}C]-AEA rat brain homogenates	IC_{50} 18,5 µM	[^{3}H]-CP 55940 rat brain membranes	Deutsch [134]
	Methyldodecyl fluorophosphonate	IC_{50} 3 nM (pH 9.0)	[^{14}C]-AEA rat brain homogenates	Ki 2,54 nM	[^{3}H]-CP 55940 rat brain membranes	Martin [135]
	Arachidonylsulfonyl fluoride	IC_{50} 0,11nM (pH 7.0)	[^{14}C]-oleamide mice brain homogenates	IC_{50} 304 nM	[^{3}H]-CP 55940 mice brain membranes	Segall [136]
	*(R)*-2-octyl γ-bromoacetoacetate	IC_{50} 2,6 µM Ki 0,8 µM (pH 9.0) IC_{50} 3,1 µM (pH 7.0)	[^{14}C]-oleamide rat liver plasma membrane extracts	-	-	Patricelli [137]
	*(E)*-6-(bromomethylene) tetrahydro-3-(1-napthtalenyl)-2*H*-pyrano-2-one (BTNP)	IC_{50} 0,8 µM (pH 8.0)	[^{3}H]-AEA rat brain homogenates	-	-	Beltramo [138]

(Table 1) contd….

Chemical structure	Name	FAAH inhibition		Affinity for CB_1 receptor		References
		Ki / IC_{50} (assay pH)	Radioligand used, source of FAAH	Ki / IC_{50}	Radioligand used, source of CB1 receptor	
	carbamic acid, cyclohexyl-3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl ester (URB 597)	IC_{50} 4,6 nM (pH not mentionned)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 100 µM	[^{3}H]-WIN 55212,2 rat cerebellar membranes	Kathuria [35]
	*N*-butylcarbamic acid –4-(phenylmethoxy) phenyl ester	IC_{50} 390 nM (pH not mentionned)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 30 µM	[^{3}H]-WIN 55212,2 rat cerebellar membranes	Tarzia [139]
	*N*-cyclohexylcarbamic acid biphenyl-3-yl ester	IC_{50} 63 nM (pH not mentionned)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 30 µM	[^{3}H]-WIN 55212,2 rat cerebellar membranes	Tarzia [139]

[144]. After the finding that potency of FAAH inhibition can show pH dependency [145], *N*-(1-oxohexadecyl)glycine methyl ester was assessed for its ability to inhibit AEA metabolism at three assay pH values. The IC_{50} values were similar at pH 6, 7.6 and 9. However, the maximum observable inhibition was greater at pH 9 (100%inhibition) than at pH 6 (75% inhibition).

Modification of the chemical structure of anandamide also provides potent inhibitors of FAAH. In 1998, arachidonoylserotonin was found to inhibit FAAH in either N18TG2 or RBL-2H3 cell membrane preparations. The compound was devoid of affinity for CB_1 receptor, and did not behave as a cannabimimetic agent in mice, as demonstrated with the lack of response in the mouse tetrad test [146]. Arachidonoyldopamine and pinolenoyldopamine are potent inhibitors of FAAH, however they are potent ligands of the CB_1 receptor with Ki values of 1 and 0, 25 µM. They are also good ligands of the CB_2 receptor, with Ki values of 11, 3 and 12 µM. Arachidonoyldopamine was evaluated *in vivo* in the mouse tetrad test, and it acts as a cannabimimetic substance which induces analgesia, inhibits spontaneous activity in an open field, decreases rectal temperature and causes immobility on a ring [58].

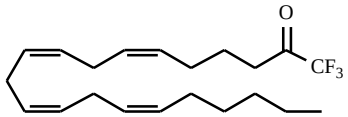
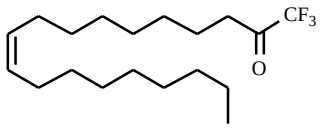
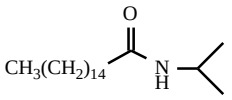
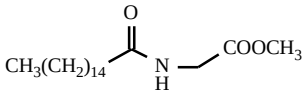
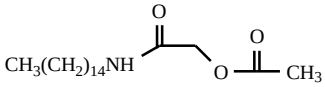
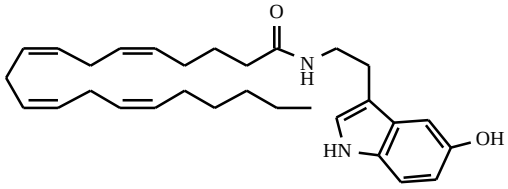
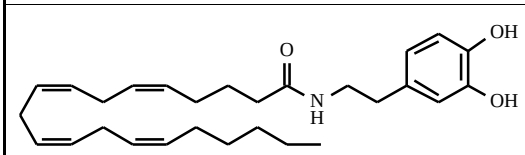
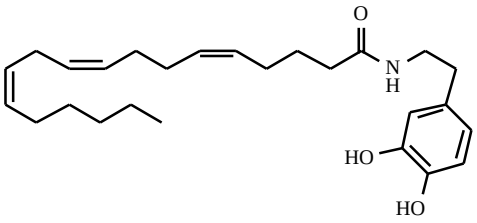
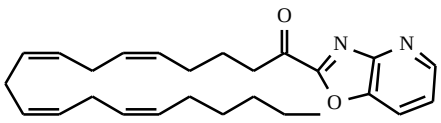
In 2000, Boger synthesised 1-oxazolo [4, 5-*b*]pyridin-2-yl eicosa-5*Z*, *8Z*, *11Z*, *14Z*-tetraen-1-one, which inhibits FAAH with a Ki value of 1 nM [147]. In 2002, van der Stelt reported the inhibition of FAAH by oxygenated metabolites of anandamide; enzymatically synthesised by soybean and barley lipoxygenases. 15(*S*)-hydroxy-eicosa-5*Z*, 8*Z*, 11*Z*, 13*E*-tetraenoyl-*N*-(2-hydroxyethyl)amine was found to inhibit FAAH with a Ki value of 0, 63 µM, and to bind selectively to the CB_1 receptor. 11(*S*)-hydroxy-eicosa-5*Z*, 8*Z*, 12*E*, 14*Z*-tetraenoyl-*N*-(2-hydroxyethyl)amine is characterised by a Ki value of 0, 57 µM and did not bind to either receptor [148].

As he did with arachidonoyl fatty acid chain, Boger synthesised oleoyl α-keto heterocycle inhibitors. 1-oxazolo [4, 5-*b*] pyridin-2-yl-9*Z*-octadecen-1-one and its methylated derivative in position 2 are potent inhibitors of FAAH with Ki values of 2, 3 and 9, 1 nM, respectively [149]. The most potent inhibitor of the α-keto heterocycle family is 1-oxazolo [4, 5-*b*] pyridin-2-yl-6-phenylhexan-1-one, characterised by a Ki value of 200 pM on rat FAAH and 94 pM on human FAAH [147]. However, the affinities of these compounds for the cannabinoid receptors were not investigated. Boger has also developed trifluoromethyl ketone inhibitors, which are not derivated of the structure of substrates of FAAH. 1, 1, 1-trifluoro-8-(2-heptylphenyl)-2-octanone and 1, 1, 1-trifluoro-9-phenyl-2-nonanone are potent inhibitors of FAAH with Ki values of 96 and 25 nM, respectively [150] (Table **2**).

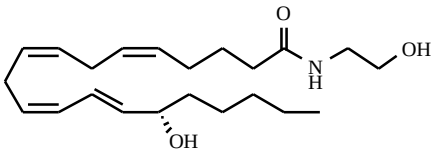
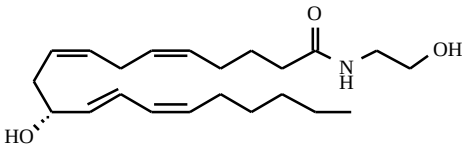
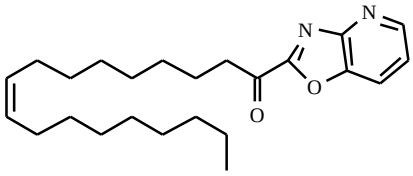
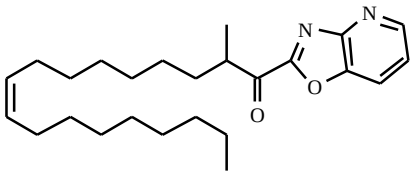
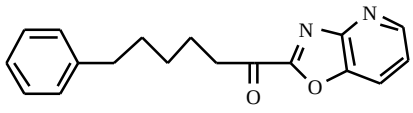
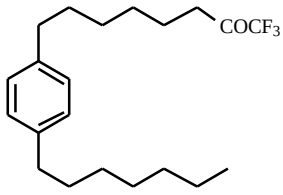
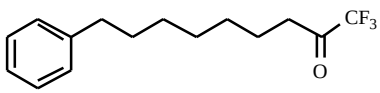
COMPOUNDS WITH INHIBITORY POTENTIAL BUT NOT DESIGNED AS INHIBITORS OF FAAH

Cannabidiol, cannabinol and Δ^9 THC inhibit FAAH activity in mouse brain microsomes. In the presence of 58 µM of anandamide, the inhibitory potency decreased from cannabidiol (160 µM, 66% inhibition), cannabinol (160 µM, 46 % inhibition) to Δ^9-THC (160 µM, 31 % inhibition) [151-154] These cannabinoids increase the Km value of FAAH without affecting Vmax, suggesting a competitive inhibition [155]. The non-steroidal anti-inflammatory drugs ibuprofen [108], [156], suprofen, ketorolac [108], flurbiprofen [156] and indomethacin [157] also produce inhibition of FAAH. The inhibitions caused by ibuprofen and ketorolac are slightly stereoselective, the *R*-isoforms of these compounds are more potent than the *S*-isoforms. In contrast, the *R*- and *S*-isoforms of flurbiprofen have similar IC_{50} values. The general anaesthetic propofol is a competitive inhibitor of FAAH characterised by a IC_{50} value of 52 µM. Propofol does not bind to the CB_1 receptor however, the CB_1 receptor

Table 2. Other Inhibitors of FAAH

Chemical structure	Name *Mechanism of inhibition*	FAAH inhibition		Affinity for CB_1 receptor		References
		Ki / IC_{50} (assay pH)	**Radioligand used, source of FAAH**	**Ki / IC_{50}**	**Radioligand used, source of CB1 receptor**	
	arachidonoyl trifluoromethyl ketone (ATFMK) *transition-state inhibitor*	IC_{50} 0,23 µM (pH 7.6)[108] IC_{50} 3 µM[99] (pH 7.4)	[^{3}H]-AEA rat brain homogenates [108] [^{14}C]-AEA N18TG2 cells homogenates [99]	Ki 0,65 µM [140]	[^{3}H]-CP 55940 rat brain membranes [140]	Koutek [140] Fowler [108] Maurelli [99]
	Oleoyltrifluoro-methyl ketone *Transition-state inhibitor*	Ki 1,2 nM (pH 10.0) Ki 0,082 µM IC_{50} 0,46 µM (pH 9.0)	oleamide rat liver plasma membrane extracts [^{14}C]-oleamide rat liver plasma membrane extracts	- -	- -	Patterson [141] Boger [150]
	Palmitoyl isopropylamide *Mixed-type inhibitor*	IC_{50} 12,8µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 100 µM	[^{3}H]-CP 55940 hCB_1 transfected CHO cells	Jonsson [142]
	*N*-(1-oxohexadecyl) glycine methyl ester *Mechanism not yet elucidated*	IC_{50} 10 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 10 µM	[^{3}H]-CP 55940 hCB_1 transfected CHO cells	Vandevoorde [144]
	*N*-(2-acetoxyacetyl) pentadecylamine *Mechanism not yet elucidated*	IC_{50} 8,3 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	No binding at concentration as high as 10 µM	[^{3}H]-CP 55940 hCB_1 transfected CHO cells	Vandevoorde [144]
	Arachidonoyl-serotonin *Non-covalent inhibition*	IC_{50} 12 µM (pH 9.0) IC_{50} 5,6 µM (pH 9.0)	[^{14}C]-AEA N18TG2 cell membranes [^{14}C]-AEA RBL-2H3 cell membranes	No binding at concentration as high as 50 µM	[^{3}H]-SR 141716A N18TG2 cell membranes	Bisogno [146]
	Arachidonoyl-dopamine *competitive*	IC_{50} 22 µM IC_{50} 23 µM	[^{14}C]-AEA N18TG2 cell membranes [^{14}C]-AEA RBL-2H3 cell membranes	Ki 0,25 µM	[^{3}H]-SR 141716A rat brain homogenates	Bisogno [58]
	Pinolenoyldopamine *Mechanism not yet elucidated*	IC_{50} 19 µM	[^{14}C]-AEA N18TG2 cell membranes	Ki 1,0 µM	[^{3}H]-SR 141716A rat brain homogenates	Bisogno [58]
	1-oxazolo[4,5-*b*]pyridin-2-yl eicosa-*5Z,8Z, 11Z,14Z*-tetraen-1-one (compound 38 of [147]) *Mechanism not yet elucidated*	Ki 1 nM (pH 9.0)	[^{14}C]-oleamide rat liver plasma membrane extracts	-	-	Boger [147]

(Table 2) contd….

Chemical structure	Name *Mechanism of inhibition*	FAAH inhibition		Affinity for CB_1 receptor		References
		Ki / IC_{50} (assay pH)	**Radioligand used, source of FAAH**	**Ki / IC_{50}**	**Radioligand used, source of CB1 receptor**	
	15(*S*)-hydroxy-eicosa-5*Z*,8*Z*, 1*Z*,13*E*-tetraenoyl-*N*-(2-hydroxyethyl) amine *Mechanism not yet elucidated*	Ki 0,63 µM (pH 9.0)	[^{3}H]-AEA U937 cells	Ki 600 nM	[^{3}H]-CP 55940 rat brain membranes	Van der Stelt [148]
	(11(*S*)-hydroxy-eicosa-5*Z*,8*Z*,12*E*,14*Z*-tetraenoyl-*N*-(2-hydroxyethyl)amine *Mechanism not yet elucidated*	Ki 0,57 µM (pH 9.0)	[^{3}H]-AEA U937 cells	Ki > 1000 nM	[^{3}H]-CP 55940 rat brain membranes	Van der Stelt [148]
	1-oxazolo[4,5-b] pyridin-2-yl-9*Z*-octadecen-1-one (compound 3 of [149]) *Mechanism not yet elucidated*	Ki 2,3 nM (pH 9.0)	[^{14}C]-oleamide rat liver plasma membrane extracts	-	-	Boger [149]
	2-methyl-1-oxazolo[4,5-b] pyridin-2-yl-9*Z*-octadecen-1-one (compound 15 of [149]) *Mechanism not yet elucidated*	Ki 9,1 nM (pH 9.0)	[^{14}C]-oleamide rat liver plasma membrane extracts	-	-	Boger [149]
	1-oxazolo[4,5-b] pyridin-2-yl-6-phenylhexan-1-one (compound 53 of [147]) *Mechanism not yet elucidated*	Ki 200 pM (pH 9.0) Ki 94 pM (pH 9.0)	[^{14}C]-oleamide rat liver plasma membrane e xtracts [^{14}C]-oleamide human FAAH transfected COS-7 cells	-	-	Boger [147]
	1,1,1-trifluoro-8-(4-heptylphenyl)-2-octanone (compound 13 of [150]) *Mechanism not yet elucidated*	Ki 96 nM IC_{50} 0,59 µM	[^{14}C]-oleamide rat liver plasma membrane	-	-	Boger [150]
	1,1,1-trifluoro-9-phenyl-2-nonanone (compound 21 of [150]) *Mechanism not yet elucidated*	Ki 25 nM IC_{50} 0,12 µM	[^{14}C]-oleamide rat liver plasma membrane	-	-	Boger [150]

antagonist SR141716A and the CB_1 receptor agonist WIN 55, 212-2 significantly potentiate the loss of righting reflex produced by propofol. The intra peritoneal administration of propofol was also shown to increase the brain content of anandamide from 19 ng/g wet tissue to 23 ng/g wet tissue [158].

FAAH$^{(-/-)}$ Mice

In 2001, Cravatt reported the generation of FAAH$^{(-/-)}$ mice, which possess enhanced endogenous cannabinoid levels and activity [201]. Brain and liver tissues from FAAH$^{(-/-)}$ mice hydrolyse anandamide and oleamide 50-100-fold more slowly than brain extracts from FAAH$^{(+/+)}$ mice. Brain levels of anandamide are also enhanced in FAAH$^{(-/-)}$ mice, which present 15-fold higher levels of anandamide than brains from FAAH$^{(+/+)}$ mice. The responses of FAAH$^{(-/-)}$ and FAAH$^{(+/+)}$ mice to anandamide were compared in the tetrad test. In FAAH$^{(-/-)}$ mice, AEA produces behavioural responses at very low doses (6.25-50 mg/kg), which are totally inactive in FAAH$^{(+/+)}$ mice. Anandamide causes hypomotility, analgesia, catalepsy and hypothermia, which are blocked by pre-treatment with the CB_1 receptor antagonist SR141716A. However, by 24h post-treatment with anandamide, FAAH$^{(-/-)}$ are indistinguishable from the FAAH$^{(+/+)}$ mice. FAAH$^{(-/-)}$ mice also exhibit reduced pain sensitivity, even in the absence of exogenous administration of a cannabinoid agent. They naturally exhibit a reduction in pain behaviour in the tail-immersion, hot plate and during the early phase of the formalin test. Furthermore, this naturally reduced pain sensitivity of FAAH$^{(-/-)}$ mice is completely inhibited by the administration of the CB_1 receptor antagonist SR141716A. Very recently, it was reported that the FAAH$^{(-/-)}$ mice exhibit a proconvulsant activity. Treatment of FAAH$^{(-/-)}$ mice with AEA significantly enhance the severity of bicuculline- or kainate- induced seizures, while AEA does not induce the same effect in FAAH$^{(+/+)}$ mice [160].

MONOGLYCERIDE LIPASE

Molecular Structure

In 1976, Tornqvist and Belfrage reported the purification of an enzyme responsible for the main monoacylglycerol hydrolysing activity of crude adipose rat tissue [161]. Enzymatic degradation of 2-AG was later demonstrated in porcine islets homogenate [162] and in circulating and tumoural macrophages [163]. In 1997, the cDNa cloning of the mouse monoglyceride lipase (MGL) was succeeded by Karlsson. The mouse amino acid sequence consists of 303 amino acids, corresponding to a molecular weight of 33, 218 daltons. The catalytic triad of this serine hydrolase was also identified to be consisted of Ser-122, Asp-239 and His-269, and was confirmed by site-mutagenesis experiments [164]. Five years later, the cDNA cloning of the rat MGL was completed by Dinh. The rat cDNA sequence encodes for a 303 amino acids protein with a molecular weight of 33, 367 daltons [78]. The human MGL cDNA was also cloned and shows 84 % identity with the mouse MGL [165] and 85 % identity with the rat MGL (Fig. **3**). The residues identified to constitute the catalytic triad of mouse MGL were found to be conserved in human MGL.

Distribution

MGL is ubiquitously distributed in rat organs. Northern blot analysis confirmed the presence of MGL mRNA in

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Mouse   MPEASSPRRTPQNVPYQDLPHLVNADGQYLFCRYWKPSGTPKALIFVSHGAGEHCGRYDE  60
Rat     MPEASSPRRTPQNVPYQDLPHLVNADGQYLFCRYWKPSGTPKALIFVSHGAGEHCGRYDE
Human   MPEESSPRRTPQS I PYQDLPHLVNADGQYLFCRYWKPTGTPKALIFVSHGAGEHSGRYE E

Mouse   LAHMLKGLDMLVFAHDHVGHGQSEGERMVVSDFQVFVRDVLQHVDTI QKDYPDVPI FLLG 120
Rat     LAQMLKRLDMLVFAHDHVGHGQSEGERMVVSDFQVFVRDL LQHVNTVQKDYPEVPVFLLG
Human   LARMLMGLDLLVFAHDHVGHGQSEGERMVVSDFHVFVRDV LQHVDSMQKDYPGLPVFLLG

Mouse   HSMGGAIS ILVAAERPTYFSGMVLISPLVLANPESASTLKVLAAKLLNFVLPNMTLGRID 180
Rat     HSMGGAIS ILAAAERPTHFSGM I LISPLI LANPESAS TLKVLAAKLLNFVLPNI S LGRID
Human   HSMGGAIAILTAAERPGHFAGMVLISPLVLANPESATTFKVLAAKVLNLVLPNLSLGPID

Mouse   SSVLSRNKSEVDLYNSDPLVCRAGLKVCFGIQLLNAVARVERAMPRLTLPFLLLQGSADR 240
Rat     SSVLSRNKSEVDLYNSDPLI CHAGVKVCFGIQLLNAVS RVERAMPRLTLPFLLLQGSADR
Human   SSVLSRNKTEVDIYNSDPL I CRAGLKVCFGIQLLNAVS RVERAL PKLTVPFLLLQGSADR

Mouse   LCDSKGAYLLMES S RSQDKTLKMYEGAYHVLHRELPEVTNSVLHEVNSWVSH RIAAAGAG 300
Rat     LCDSKGAYLLMES S PSQDKTLKMYEGAYHVLHK ELPEVTNSVLHEINTWVSH RI AVAGAR
Human   LCDSKGAYLLMELAKSQDKTLK I YEGAYHVLHK ELPEVTNSVFHEI NMWVSQRTATAGTA

Mouse   CPP 303
Rat     CLP 303
Human   SPP 303
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Fig. (3). Amino acid sequence alignment between mouse, rat and human MGL. Identical amino acids are shaded in gray. The residues constituting the catalytic triad are boxed.

adipose tissue, adrenal gland, heart, brain, kidney, testis, ovary, spleen, lung, liver and skeletal muscle [164]. In situ hybridisation revealed that MGL mRNA is heterogeneously expressed in the rat brain, with highest levels in regions where CB_1 receptors are also present (hippocampus, cortex, anterior thalamus and cerebellum) [78].

Assays

To investigate the nature of the substrates hydrolysed by MGL, Dinh *et al.* have overexpressed MGL in HeLa cells by adenoviral vector-mediated gene transfer [78]. This method confers high levels of MGL immunoreactivity to these cells, which normally do not express MGL. They incubated supernatant protein in sodium phosphate buffer (50 mM, pH 8.0) with 2-oleoyl- [^{3}H]-glycerol or 2- [^{3}H]-AG as substrates. The reaction products were separated by organic solvent extraction (chloroform:methanol, 1:1). When 2-oleoyl- [^{3}H]-glycerol was used, the released [^{3}H]-glycerol in the aqueous phase was counted by scintillation counting. When 2- [^{3}H]-AG was used, the organic phase was subjected to a further TLC purification.

Substrates

Dinh *et al.* found that MGL was capable of hydrolysing 2-AG and 2-oleoyl-glycerol, a result, which was earlier reported by Tornqvist on a purified enzyme from rat adipose tissue [161]. The question of the possible hydrolysis of AEA by MGL was particularly relevant, since FAAH hydrolyses AEA and 2-AG *in vitro* [112]. Goparaju even reported that the hydrolysis of 2-AG by FAAH proceeds about 4-fold faster than the anandamide hydrolysis [112]. However, several observations indicated that the hydrolysis of 2-AG proceeds by another enzyme, different of FAAH. A rapid degradation of 2-AG was observed in mouse blood, under the conditions where anandamide is stable [166]. The $FAAH^{(-/-)}$ which is characterised by a total loss of AEA hydrolysing activity, preserves its capacity to hydrolyse 2-AG [167]. However, MGL and FAAH are different in terms of substrate specificity and MGL preferentially hydrolyses 2-monoglycerides but not fatty acid ethanolamides such as AEA and PEA [78].

Inhibitors

MGL activity is inhibited by various serine hydrolase inhibitors that were previously shown to block FAAH. Interestingly, the IC_{50} values observed are higher for the inhibition of MGL hydrolysis than those obtained for inhibition of FAAH hydrolysis. Indeed, arachidonoylfluorophosphonate(MAFP), arachidonoyl trifluoromethylketone (ATFMK) and hexadecylsulfonylfluoride(AM374) are characterised by IC_{50} values for FAAH of 3 nM, 0, 23 μM and 10, 2 nM and IC_{50} values for MGL of 0, 8 μM, 2, 5 μM and 6, 2 μM [159], respectively.

In 1998, Ben-Shabat reported the coexistence, in the spleen, brain and gut, of 2-AG and several inactive endogenous fatty acid glycerol esters, like 2-linoleoylglycerol and 2-palmitoylglycerol. These esters are devoid of affinity for the CB receptors, however they significantly potentiate the CB binding, immobility, analgesia and hypothermia caused by 2-AG in mice. These compounds were described as responsible of an "entourage effect" capable of regulating the 2-AG cannabinoid activity. The inhibitory potential of the two esters on 2-AG hydrolysis was tested using mouse neuroblastoma N18TG2 and rat basophilic leukaemia cells, previously shown to contain enzymatic activities for catalysing 2-AG hydrolysis to arachidonic acid. 2-linoleoylglycerol but not 2-palmitoylglycerol, was shown to inhibit the enzymatic degradation of 2-AG in these cell lines [63]. However, the enzyme responsible of the degradation of 2-AG in these cell lines rather appears to be FAAH since the hydrolysis of 2-AG was inhibited by AEA [168].

N-PALMITOYLETHANOLAMINE-SELECTIVE ACID AMIDASE

Distribution-Substrates

In 1999, Ueda *et al.* discovered a new hydrolase, distinguishable from FAAH, in human megakaryoblastic cell line (CMK). This enzyme was present in the 12000 X g pellet of the CMK cell homogenate and was solubilised by freeze-thaw. PEA was the most active substrate for this solubilised enzyme, which was christened "*N*-palmitoylethanolamine selective acid amidase" or "NPAA". This enzyme was characterised by a molecular weight of 31 kDa, an optimal acidic pH of 5 [79] and it was activated by triton X-100. Two years later, the same group succeeded the isolation of NPAA from different rat tissues. The higher activity was found in the lung, spleen, small intestine, thymus, caecum and in peritoneal and alveolar macrophages. Lower activity was detected in the brain, submaxillary gland, heart, stomach, liver, large intestine, kidney and testis [169]. Very recently, NPAA activity was detected in the cytosol of RBL-1 cells whereas FAAH activity was found in the particulate fraction of these cells [170].

The purified NPAA lung enzyme was allowed to react with various *N*-acylethanolamides. PEA was found to be the most active substrate followed by *N*-myristoylethanolamide (C14:0), *N*-stearoylethanolamide(C18:0), *N*-oleoylethanolamide(C18:1), *N*-linoleoylethanolamide(C18:2) and finally AEA, which was poorly hydrolysed by NPAA [76].

Assays

Ueda *et al.* developed a NPAA assay in which the 12000 X g pellet of rat lung homogenates are incubated in presence of [^{14}C]-PEA in a citrate-sodium phosphate buffer (pH 5.0) containing DTT and Triton X-100. The reaction is terminated by addition of a mixture of diethyl ether/methanol/citric acid and the ethereal extracts are separated on a silica gel sheet eluated with a mixture of chloroform/methanol/28% ammonium hydroxide before counting of the radioactivity [170].

Inhibitors

NPAA is almost insensitive to PMSF and MAFP, two potent inhibitors of FAAH. Because PEA is the most active substrate of NPAA, derivatives of palmitic acid including esters, amides, retroesters and retroamides have been investigated as NPAA inhibitors. This has allowed the discovery of cyclohexylpalmitate and *N*-(3-hydroxypropionyl)-pentadecanamide (Fig. **4**). These compounds induce

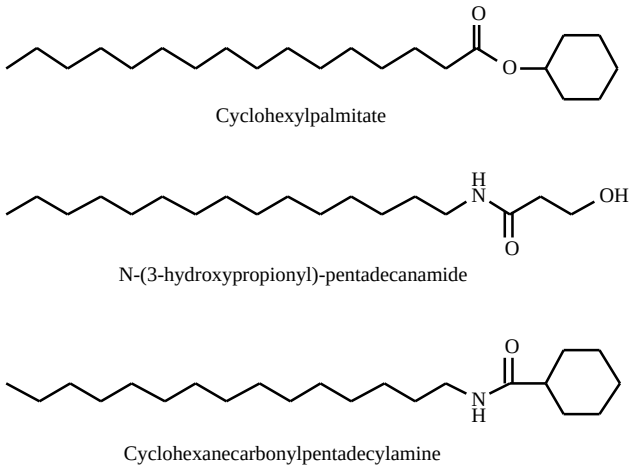


Fig. (4). Structures of cyclohexylpalmitate, *N*-(3-hydroxypropionyl)-pentadecanamide and *N*-cyclohexanecarbonylpentadecylamine, three inhibitors of NPAA.

84 and 77 % inhibition of NPAA at 100 µM (in presence of 100 µM [^{14}C]-PEA) and they are characterised by IC_{50} values of 19 and 31 µM, respectively. Moreover, they are devoid of CB_1 and CB_2 affinity and they do not alter FAAH activity [171]. *N*-cyclohexanecarbonylpentadecylamine (Fig. **4**) is a more potent inhibitor, which causes 95 % inhibition of NPAA and is characterised by a IC_{50} value of 4.5 µM. The selectivity of the compound for NPAA has allowed its use as a tool to distinguish NPAA from FAAH, in RBL-1 cells [170].

UPTAKE PROCESS: A NECESSARY STEP OF THE ENDOCANNABINOIDS DEGRADATION

If FAAH, MGL and NPAA are the subject of a growing interest which has allowed an extensive characterisation and an in-depth knowledge of these three enzymes, less is known however about the way taken by their substrates, the endocannabinoids, to reach their catalytic sites. In this review, our intention is not to make an extensive description of the endocannabinoids transporters. This subject was already covered by four recent reviews of Hillard [172, 173], Giuffrida [174] and Fowler [175]. However, the quest for inhibitors of the endocannabinoids metabolism could not be carried out without considering their cellular transport to the catalytic sites of the enzymes responsible of their degradation. We propose to highlight the different observations, which prove or, on the contrary, invalidate the existence of endocannabinoids transporters. We also consider interesting to list the developed inhibitors of this major, but not yet cloned, putative uptake protein.

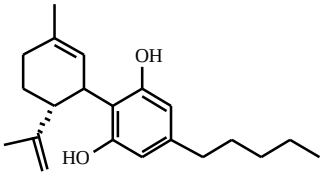
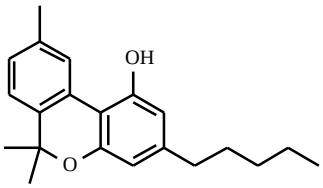
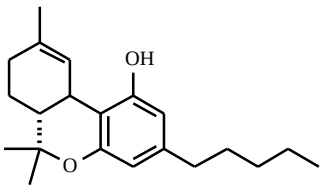
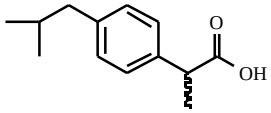
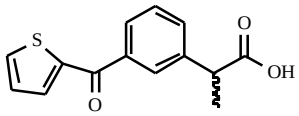
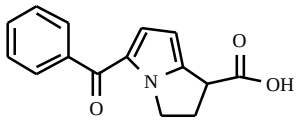
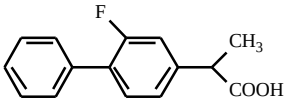
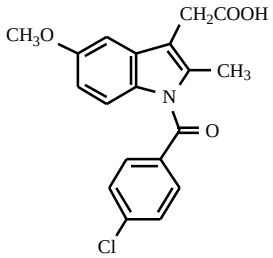
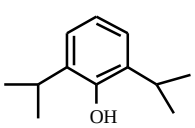
Anandamide Uptake

In 1994, Di Marzo *et al.* published the first report of a saturable and temperature-sensitive uptake of AEA with the characteristics of a facilitated diffusion. This first cellular transport of AEA was observed in cortical granule neurons and was rapidly followed by the hydrolysis of AEA by FAAH [176]. Three years later, Hillard *et al.* reported a time- ($t_{1/2}$ = 2.6 min at 37°C) and temperature-dependent accumulation of AEA in cerebellar granule cells. This accumulation was saturable and characterised by an apparent K_m of 41 µM and a V_{max} value of 0.61 nmol/min/10^6 cells and was shown to be independent of sodium gradient and ATP. The observed accumulation was selective for AEA: only oleoylethanolamide was able to inhibit the cellular accumulation of AEA, at high concentration, while palmitoylethanolamide and linolenoylethanolamide were inactive [177]. Other investigators also demonstrated the existence of an AEA uptake process in cortical astrocytes [178], C6 glioma cells [74], N18TG2 neuroblastoma cell lines [74], CHP100 cells [102], human astrocytoma CCF-STTG1 cell line [179] and endothelial cells [180].

In 2000, Rakhshan *et al.* investigated the hypothesis that the uptake process in peripheral cells could be similar to that previously observed in the central nervous system. They showes that the mast cell line RBL-2H3 also exhibited a time- ($t_{1/2}$ = 3.2 min at 37°C) and temperature-dependent accumulation of AEA. This accumulation was saturable and characterised by an apparent K_m of 11.4 µM and a V_{max} value of 0.17 nmol/min/10^6 cells and was shown to be independent of Na^+, Cl^- and H^+ gradients. Uptake process in RBL-2H3 cells was essentially selective for AEA: palmitoylethanolamide, ethanolamine, stearic acid and maleic acid were unable to inhibit the AEA accumulation in these cells. Interestingly, 2-AG, arachidonic acid and oleic acid elicitated a dose-dependent decrease in anandamide uptake in RBL-2H3 cells [181]. Cellular accumulation of AEA was also found to occur in J774 macrophages [99] and lymphoma U937 cell line [102]. However, it must be mentioned that all these cells do not accumulate AEA to the same degree. The uptake of AEA was the most effective in lymphoma U937 cell line and the lowest in human astrocytoma CCF-STTG1 cell line.

The mechanism of AEA cellular transport remains to be elucidated, however, the lack of dependence of ATP and sodium gradient suggest that AEA uptake is driven by diffusion and does not require energy. In 2000, Maccarrone *et al.* [180] reported the stimulation of AEA uptake in human

Table 3. Compounds with Inhibitory Potential but not Designed as Inhibitors of FAAH

Chemical structure	Name *Mechanism of inhibition*	FAAH inhibition		Affinity for CB_1 receptor		References
		Ki / IC_{50} (assay pH)	Radioligand used, source of FAAH	Ki / IC_{50}	Radioligand used, source of CB1 receptor	
	Cannabidiol *competitive*	66 % inhibition at 160 µM	58 µM AEA rat brain homogenates (AEA not radioactive, GC analysis)	Ki > 10 µM [152]	[^{3}H]-HU 243 rat brain homogenates [152]	Watanabe [151] Bisogno [152]
	Cannabinol *Mechanism not yet elucidated*	46 % inhibition at 160 µM	58 µM AEA rat brain homogenates (AEA not radioactive, GC analysis)	Ki 211 nM [153]	[^{3}H]-HU 243 rat CB_1transfected COS-7 cells	Watanabe [151] Rhee [153]
	Δ^9 THC *Mechanism not yet elucidated*	31 % inhibition at 160 µM	58 µM AEA rat brain homogenates (AEA not radioactive, GC analysis)	Ki 35,3 nM [154] Ki 80,3 nM [153]	[^{3}H]-CP 55,940 rat brain homogenates [154] [^{3}H]-HU 243 rat CB_1 transfected COS-7 cells[154]	Watanabe [151] Rinaldi-Carmona [154] Rhee [153]
	Ibuprofen *Mixed-type*	*R*- : IC_{50} 230 µM (pH 7.6) *S*- : IC_{50} 750 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	-	-	Fowler [156]
	Suprofen *Mechanism not yet elucidated*	IC_{50} 170 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	-	-	Fowler [108]
	Ketorolac *Mechanism not yet elucidated*	*R*- : IC_{50} 50 µM (pH 7.6) *S*- : IC_{50} 440 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	-	-	Fowler [156]
	Flurbiprofen *Mechanism not yet elucidated*	*R*- : IC_{50} 60 µM (pH 7.6) *S*- : IC_{50} 50 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates	-	-	Fowler [156]
	Indomethacin *Competitive*	Ki 120 µM (pH 7.6) Ki 330 µM (pH 7.6)	[^{3}H]-AEA rat brain homogenates [^{3}H]-AEA chicken brain homogenates	-	-	Fowler [157]
	Propofol *Competitive*	IC_{50} 52 µM (vehicle is DMSO) IC_{50} 14 µM (vehicle is intralipid)	[^{14}C]-AEA rat brain homogenates	No binding at concentration as high as 100 µM	[^{3}H]-CP 55,940 rat brain homogenates	Patel [158]

umbelical vein endothelial cells (HUVECs) by sodium nitroprusside, *S*-nitroso-*N*-acetylpenicillamine and spermine NONOate((Z)-1-{*N*- [3-aminopropyl]-*N*- [4-(3-aminopropyl-ammonio)-butyl]-amino}-diazen-1-ium-1, 2-diolate), three nitric oxide donors. Their results suggested that anandamide uptake in HUVECs is regulated by nitric oxide. Hillard *et al.* reported that cerebellar granule cells preloaded with [^{3}H]-AEA exhibit a time- and temperature-dependent AEA efflux [172]. This observation was consistent with a protein carrier capable of moving AEA in both directions across the membrane, depending on the AEA concentration gradient across the membrane. Surprising is the finding that AEA concentrations on both sides of the cell are not equal when the AEA accumulation in cerebellar granule cells reaches a steady stade [172]. This observation suggests that only a part of AEA accumulated in the cell is intact and free to equilibrate with the extracellular AEA. Many biological actors are conceivable to explain this difference: FAAH could regulate the amounts of *intact* AEA in the cell, or some membranous compartments or intracellular proteins could reduce the amounts of *free* AEA in the cell after sequestration or binding.

In 2001, Day *et al.* and Deutsch *et al.* demonstrated the important role of FAAH in the cellular uptake of AEA. The accumulation of AEA was reported in RBL-2H3 [182], N18TG2 [183] and C6 [142] cell lines, which natively express FAAH. Similarly, FAAH-HeLa [182] and FAAH-Hep2 [183] transfected cells exhibit an AEA uptake 2-fold greater than in untransfected cells that lack FAAH. MAFP, a FAAH inhibitor structurally related to AEA, partially reduces the AEA accumulation in RBL-2H3, N18TG2 and C6 cell lines, but also in wild-type HeLa cells. Interestingly, MAFP does not inhibit the AEA uptake in wild-type Hep2 cells that lack FAAH [183] nor in cortical neurons, which exhibit FAAH activity [35]. Taken together, these results suggest that FAAH-transfection to a cell enhances the uptake of AEA however; complete inhibition of FAAH only results in partial AEA uptake inhibition. These observations support a partial, but not indispensable, role of FAAH in AEA uptake process.

In their last review [173], Hillard and Jarrahian published an elegant discussion about a possible reversible binding of AEA to an intracellular site which could have the characteristics of a carrier in terms of saturability and inhibition by structurally related molecules. Moreover, the existence of an AEA transporter was recently called into question by Glaser *et al.* who performed a study at initial rates of AEA accumulation in N18TG2 neuroblastoma and CCF-STTG1 astrocytoma cell lines. In these conditions, they demonstrated that AEA uptake was not saturable and that AM404 (*N*-(4-hydroxyphenyl) arachidonylamide) did not inhibit it. However, they used a procedure quite different of the other searchers teams by adding bovine serum albumin in the medium of the uptake assay. The presence of bovine serum albumin could induce major modifications in the transport of AEA in aqueous compartments by binding to AEA [184]. Very recently, Fasia *et al.* reported AEA accumulation in rabbit platelets, in contrast to human platelets [186], was not saturable and not temperature-dependent. Moreover, they demonstrated that PMSF and ATFMK, two FAAH inhibitors, had no effect and they concluded that AEA accumulation in rabbit platelets occurs by simple diffusion [186].

Inhibitors of Anandamide Uptake

We have previously shown that modifications of the chemical structure of substrates of FAAH have allowed the discovery of potent inhibitors of this enzyme. The same strategy was helpful for the design of inhibitors of AEA uptake. However, it is difficult, by this way, to synthesise selective inhibitors of AEA uptake, without any activity against FAAH. *N*-(4-hydroxyphenyl)-arachidonamide (AM404), a compound devoid of CB_1 affinity [178], is a

Table 4. Summary of the Available Data on Fatty Acid Amide Hydrolase (FAAH), Monoglyceride Lipase (MGL) and *N*-Palmitoylethanolamine-Selective Amidase (NPAA)

	FAAH	MGL	NPAA
Localisation	brain, testis, uterus, kidney, liver	ubiquitous	lung, spleen, small intestine, thymus
Cloned ?	Yes human, rat, mouse, pig	Yes human, rat, mouse	no
Optimal pH	8.5 - 10	8	5
Substrates	AEA, *N*-oleoylethanolamine	2-AG, 2-oleoylglycerol	PEA, *N*-myristoylethanolamine, *N*-stearoylethanolamine
Potent inhibitors	1-oxazolo[4,5-b]pyridin-2-yl-6-phenylhexan-1-one ATFMK MAFP	ATFMK, MAFP, AM374	Cyclohexylpalmitate, *N*-(3-hydroxypropionyl) pentadecanamide, cyclohexanecarbonyl-pentadecylamine
Knock-out	yes	no	no

potent inhibitor of AEA accumulation in rat neurons [177], cerebellar granule cells [187] and human CCF-STTG1 astrocytoma cells [178]. However, AM 404 inhibits FAAH activity in rat forebrain membranes [187] and N18TG2 cells [188] with IC_{50} values of 0, 5 and 22 μM, respectively. It was also reported that AM404 elevates intracellular Ca^{2+} in Madin Darby canine kidney (MDCK), Chang liver, PC3 human prostate cancer, BFTC human bladder cancer and MG63 human osteoblast-like cells at a lower concentration than those commonly used to block AEA uptake [189]. This finding raised the question of the real selectivity of this compound and that caution must be exercised when this compound is used as a selective pharmacological tool to investigate the AEA transporter. *In vivo*, AM404 was shown to potentiate the hypotensive effects of AEA in anaesthesized guinea pigs [190]. *N*-(2-hydroxyphenyl)-arachidonamide, the ortho analogue of AM 404, is a less potent inhibitor of uptake than AM 404 [187]. Nevertheless, the suppression of the phenolic ring to afford arachidonamide maintains a moderate inhibition of AEA uptake, characterised by a IC_{50} value of 9 μM [179]. VDM 11, a methylated derivative of AM404, is a very selective inhibitor of AEA uptake in RBL-2H3 and C6 glioma cells. VDM 11 is devoid of vanilloid and CB_1 receptors affinity and does not exhibit inhibitory potential against FAAH [188]. AEA accumulation in RBL-2H3 and C6 glioma cells is also reduced by the two potent vanilloid agonists arvanil and olvanil [188] which do not inhibit FAAH [191, 192] but also exhibit moderate affinity for the CB_1 receptor [191, 193].

In 2001, López-Rodríguez *et al*. reported the synthesis of *N*-(3-furylmethyl) arachidonamide (UCM 707), the most potent and selective AEA transporter inhibitor described to date [194]. This compound inhibits AEA accumulation in human lymphoma U937 cells with an IC_{50} value of 0, 8 μM. UCM 707 is totally devoid of affinity for the vanilloid (Ki > 5 μM) and CB_1 receptors (Ki = 4, 7 μM), and does not inhibit FAAH [195, 196] (IC_{50} of 30 μM). However, UCM 707 exhibits remarkable affinity for CB_2 receptor (Ki = 67 nM). UCM 707 was evaluated *in vivo* in the open-field and hot-plate test. The compound was mostly inactive when administrated alone, but it was able to potentiate the hypokinetic and antinociceptive effects of AEA [197]. In 2003, Ortar *et al.* synthesised OMDM-1, a derivative of oleoylethanolamide which inhibit AEA accumulation in RBL-2H3 cells with a Ki of 2, 4 μM. This compound was inactive as CB_1 (Ki 12, 1 μM) and vanilloid (EC_{50} > 10 μM) ligand, as well as on FAAH (Ki > 50 μM) [198].

As we have shown, most of the uptake inhibitors are derived from the structures either of AEA, or of oleoylethanolamide. We investigated the ability of derivatives of palmitoylethanolamide to inhibit AEA uptake in RBL-2H3 and C6 glioma cells. We found that palmitoylcyclohexamide induces a moderate inhibition of AEA uptake with no effect on FAAH and on cannabinoid receptors [142]. This result suggests that palmitoylcyclohexamide may act as a template for the design of potent and selective inhibitors of AEA accumulation. Endogenous compounds can also act as AEA uptake inhibitors: it was reported that glutamate and glutamine inhibit AEA uptake in human, rat and mouse synaptosomes by a non-competitive manner [199].

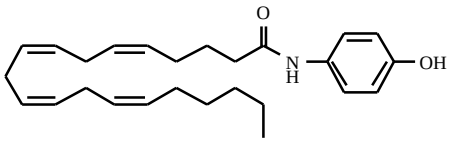
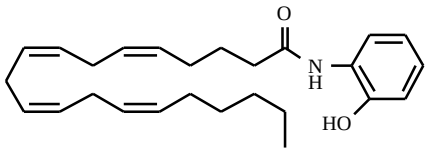
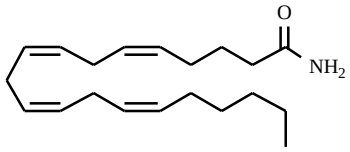
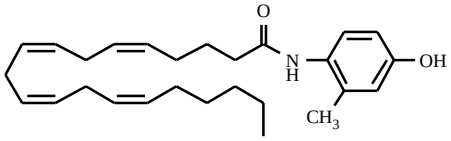
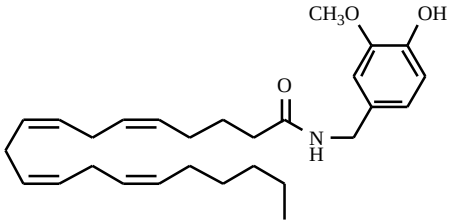
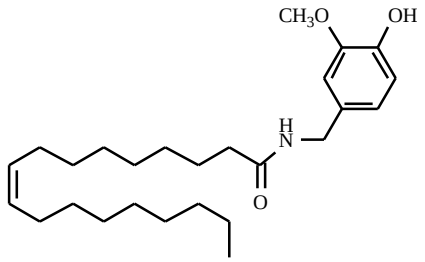
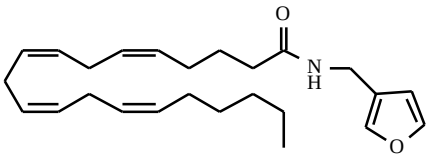
Palmitoylethanolamide Uptake

In 1997, Bisogno *et al.* published the first report on a saturable and temperature-dependent uptake of PEA and AEA in J774 macrophages and RBL-2H3 cells. The uptake of the two compounds in these cell lines was followed by hydrolysis. Because high concentrations of PEA and AEA were unable to inhibit the accumulation of, respectively, AEA and PEA, the existence of distinct uptake processes was suggested by the authors [100]. In 2001, Jacobsson and Fowler reported that a saturable and temperature-dependent facilitated transport of PEA, distinguishable from AEA uptake, occurs in Neuro-2a and RBL-2H3 cells [200]. In the same cell line, PEA uptake was characterised by higher apparent Km values than AEA uptake: apparent Km values were 10 (Neuro-2a) and 9.3 μM (RBL-2H3) for AEA uptake, and 28 (Neuro-2a) and 30 μM (RBL-2H3) for PEA uptake. Differences were also picked out in the inhibitors of these processes. AEA uptake in both cell lines was inhibited by AM404, 2-AG, methanandamide, arachidonic acid, olvanil and indomethacin but not by PEA. PEA uptake was inhibited by AEA, 2-AG, arachidonic acid, Δ^9-THC, cannabidiol but not by indomethacin. Moreover, *R1*-methanandamide only blocked PEA uptake in RBL-2H3, but not in Neuro-2a.

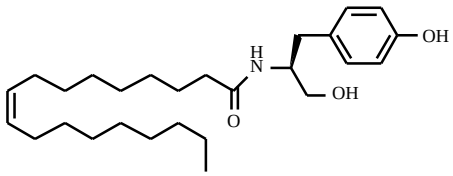
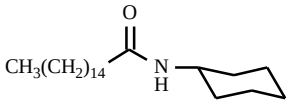
2-Arachidonoylglycerol Uptake

In 1998, Di Marzo *et al.* reported the first observation of a diffusion of 2-AG into RBL-2H3 and N18TG2 cells, with no evidence for a facilitated-diffusion process [168]. One year later, they demonstrated that [^{3}H]-2-AG is accumulated in J774 macrophages and they recovered the radioactivity in esterified [^{3}H]-2-AG to phospholipids, diacylglycerols and triglycerides [164]. Beltramo and Piomelli confirmed this observation in CCF-STTG1 astrocytoma cell line in which 2-AG is accumulated through a sodium and energy-independent process [201]. As it was reported in J774 macrophages, 2-AG is accumulated in CCF-STTG1 in an esterified form, as phospholipids, diacylglycerols and triacylglycerol, but also as free fatty acid (arachidonate) and unmetabolised 2-AG. However, the 2-AG uptake in CCF-STTG1 cells was inhibited by anandamide and AM 404, suggesting that 2-AG is uptaken by the same transporter than AEA. In 2001, Bisogno *et al.* studied simultaneously the facilitated transport of AEA and 2-AG in C6 glioma cells [202]. They found that the AEA and 2-AG uptake mechanisms in these cells are characterised by similar Km values (Km 11 and 15 μM, respectively) and different Bmax values (Bmax 1.70 and 0.24, respectively). AEA uptake in C6 cells was inhibited by 2-AG at high concentration (Ki 30 μM) while 2-AG uptake was inhibited by either 2-AG (Ki 19 μM) and AEA (Ki 20 μM). AM 404 inhibited either AEA or 2-AG uptake (Ki 7.5 and 10 μM, respectively). Sodium nitroprusside, *S*-nitroso-*N*-acetylpennicilamine and 3-morpholino-sydnonimine, three NO donors, slightly enhanced AEA and 2-AG uptake (approximatively 115% of control). These data suggested the existence of a 2-AG transporter, distinct from the AEA transporter, which shares similar molecular and regulatory features with the AEA transporter. During the same year, Maccarone *et al.* demonstrated the existence of a specific 2-AG transporter in human platelets [203]. They demonstrated

Table 5. Inhibitors of Anandamide Uptake

Chemical structure	Name	Uptake inhibition		Inhibition of FAAH	Affinity for CB_1 receptor	References
		Ki / IC_{50}	Strain used in uptake assay	Ki / IC_{50}	Ki / IC_{50}	
	*N*-(4-hydroxyphenyl)-arachidonamide AM 404	IC_{50} 3,4 µM [187] IC_{50} 2,2 µM [179]	Cerebellar granule cells Human CCF-STTG1 astrocytoma cells	IC_{50} 0,5 µM (rat brain membranes) [187] IC_{50} 22 µM (N18TG2 cells) [188]	Ki > 1 µM [189]	Jarrahian [187] Piomelli [179] De Petrocellis [188]
	*N*-(2-hydroxyphenyl)-arachidonamide	IC_{50} 5,8 µM [187]	Cerebellar granule cells	IC_{50} 2,5 µM (rat brain membranes) [187]	Ki > 1 µM	Jarrahian [187]
	Arachidonamide	IC_{50} 9,0 µM [179]	Human CCF-STTG1 astrocytoma cells	-	-	Piomelli [179]
	*N*-(2-methyl-4-hydroxyphenyl)-arachidonamide VDM 11	IC_{50} 10,2 µM IC_{50} 11,2 µM	C6 glioma cells RBL-2H3 cells	IC_{50} > 50 µM (N18TG2 cells)	Ki > 5 µM	De Petrocellis [188]
	Arvanil	IC_{50} 11,2 µM [188] IC_{50} 3,6 µM [188]	C6 glioma cells RBL-2H3 cells	IC_{50} 32 µM [191] (N18TG2cells)	Ki 0,5-2,6 µM [192]	De Petrocellis [188] Melck[191] Di Marzo[192]
	Olvanil	IC_{50} 19,9 µM [188] IC_{50} 9 µM [188]	C6 glioma cells RBL-2H3 cells	IC_{50} 48 µM [193] (RBL-2H3 cells)	Ki 1,64-7,08 µM [193]	De Petrocellis [188] Di Marzo[193]
	*N*-(3-furylmethyl) arachidonamide UCM 707	IC_{50} 0,8 µM [194]	Human lymphoma U937 cells	IC_{50} 30 µM [195, 196] (rat brain membranes)	Ki 4,7 µM [195, 196]	López-Rodríguez [194-196]

(Table 5) contd….

Chemical structure	Name	Uptake inhibition		Inhibition of FAAH	Affinity for CB_1 receptor	References
		Ki / IC_{50}	**Strain used in uptake assay**	**Ki / IC_{50}**	**Ki / IC_{50}**	
	OMDM-1	Ki 2,4 µM	RBL-2H3 cells	Ki > 50 µM (rat brain homogenates)	Ki 12, 1 µM	Ortar [198]
	Palmitoyl-cyclohexamide	AEA uptake reduced to 76 %	C6 glioma cells	No inhibition at 100 µM	No binding as concentration as high as 100µM	Jonsson [142]

that human platelets accumulate 2-AG through a transporter (Km 300 nM). Since AEA and AM404 did not inhibit 2-AG uptake, these results suggest that 2-AG is uptaken in platelets through a transporter distinguishable of the AEA transporter.

CONCLUSION

It is now established that the endocannabinoids have, by their actions on the cannabinoid receptors, a number of interesting pharmacological properties including antinociception, modulation of feeding, neuroprotection and antiproliferative effect. However, their therapeutic use is strongly prevented by their rapid hydrolysis. There is thus a need of potent inhibitors of endocannabinoid degradation. Ten years after the first observation of an enzyme responsible of endocannabinoid hydrolysis, huge progresses have been accomplished in the characterisation of these enzymes. Three enzymes have now been identified, namely fatty acid amide hydrolase, monoglyceride lipase and *N*-palmitoylethanolamine-selective amidase. Their remarkable selectivity for the major actors of the endocannabinoid signalling suggest that the endocannabinoid system is a complex machinery in which the function of each component is dependent of the others. AEA and 2-AG may activate the cannabinoid receptors in the extracellular medium and when the CB activation is no longer required, be uptaken into the cell to be hydrolysed. PEA does not bind to the CB receptors but it can act as an endogenous regulator of the AEA and 2-AG levels by "entourage effect" or bind to a specific receptor, which is not yet known. Nevertheless, evidences now exist that *in vivo* inhibition of the endocannabinoid degradation leads to antinociception, hypotension and modulation of anxiety. These results prove that inhibition of endocannabinoid degradation is a promising way in the discovery of new medicines.

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