A Review on the Monoacylglycerol Lipase: At the Interface Between Fat and Endocannabinoid Signalling

Article *in* Current Medicinal Chemistry · August 2010

Impact Factor: 3.85 · DOI: 10.2174/092986710791859414 · Source: PubMed

3 authors, including:

Johan Wouters
University of Namur
297 PUBLICATIONS **3,653** CITATIONS
SEE PROFILE

Didier M Lambert
Université catholique de Louvain
179 PUBLICATIONS **4,873** CITATIONS
SEE PROFILE

A Review on the Monoacylglycerol Lipase: At the Interface Between Fat and Endocannabinoid Signalling

G. Labar[1,2], J. Wouters[2] and D.M. Lambert*[,1]

[1]*Université catholique de Louvain, Louvain Drug Research Institute, Cannabinoid and endocannabinoid research group, Pharmaceutical Chemistry Dpt, Avenue E. Mounier 73.40, 1200 Brussels, Belgium*

[2]*Facultés universitaires Notre-Dame de la Paix, Faculté des Sciences, Laboratoire de Chimie biologique structurale, rue de Bruxelles 61, 5000 Namur, Belgium*

Abstract: Together with anandamide, 2-arachidonoylglycerol (2-AG) constitutes one of the main representatives of a family of endogenous lipids known as endocannabinoids. These act by binding to CB_1 and CB_2 cannabinoid receptors, the molecular target of the psychoactive compound Δ^9-THC, both in the periphery and in the central nervous system, where they behave as retrograde messengers to modulate synaptic transmission.

These last years, evidence has accumulated to demonstrate the lead role played by the monoacylglycerol lipase (MAGL) in the regulation of 2-arachidonoylglycerol (2-AG) levels. Considering the numerous physiological functions played by this endocannabinoid, MAGL is now considered a promising target for therapeutics, as inhibitors of this enzyme could reveal useful for the treatment of pain and inflammatory disorders, as well as in cancer research, among others.

Here we review the milestones that punctuated MAGL history, from its discovery to recent advances in the field of inhibitors development. An emphasis is given on the recent elucidation of the tridimensional structure of the enzyme, which could offer new opportunities for rational drug design.

Keywords: 2-arachidonoylglycerol, endocannabinoid system, monoacylglycerol lipase, monoglyceride lipase, inhibitor.

1. INTRODUCTION

Cannabis sativa has a long history of recreative and medicinal use. Its capacity to soothe pain and anxiety was already reported by Chinese and Indian civilizations more than three thousands years ago [1]. However, the modern history of cannabis started in 1964, when Mechoulam and Gaoni elucidated the structure of the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) [2].

This finding, as well as the observation that the biological activity of THC and other related compounds (referred to as cannabinoids) was strongly stereochemistry-dependent constituted clear signs of a receptor-driven mechanism [3,4]. In 1990, definite evidence for the existence of an endogenous receptor for Δ^9-THC came when an orphan G-protein coupled receptor (GPCR) binding cannabinoids with the expected characteristics -affinity, stereoselectivity, coupling to Gi protein- was cloned and named CB_1 cannabinoid receptor [5]. Shortly after, a second, peripheral cannabinoid receptor was cloned, the CB_2 cannabinoid receptor [6]. Meanwhile, endogenous lipids were detected in various biological samples and reported to bind and activate the recently discovered receptors, thereby eliciting several of the pharmacological properties of cannabinoids. The first two representatives of this new family of lipid transmitters (termed endocannabinoids) to be identified were *N*-arachidonoylethanolamine (anandamide) [7] and 2-arachidonoylglycerol (2-AG) [8,9], which are still considered the main actors of the endocannabinoid system (Fig. (**1**)).

*Address correspondence to this author at the Université catholique de Louvain, Louvain Drug Research Institute, Cannabinoid and endocannabinoid research group, Pharmaceutical Chemistry Dpt, Avenue E. Mounier 73.40, 1200 Brussels, Belgium; Tel: +32 2 764 73 47; Fax: + 32 2 764 73 63; E-mail: Didier.lambert@uclouvain.be

The purpose of the endocannabinoid system is to mediate an adaptative response to different pathological stimuli and to regulate fundamental physiological functions [10,11]. This pleiotropy of actions is allowed by the ubiquitous distribution of the actors of the system, and by the multiplicity of signal transduction pathways which are triggered when the receptors are stimulated [12]. The effects of endocannabinoids are mediated both at the central level and in the periphery. In the central nervous system, the CB_1 receptor-mediated modulation of ionic currents and synaptic plasticity plays a major – although not exclusive – contribution to diverse processes such as the control of memory, learning, pain, appetite, psychoaffective state, movement, addiction and neuroprotection. In the periphery, other roles include the regulation of metabolism, pain, inflammation, cell proliferation, reproductive and cardiovascular functions, among others [13,14].

Several biosynthesis and degradative processes govern the subtle balance between the production and the degradation of endocannabinoids [15-17] (Fig. (**1**) and reference [18] for a general review on the endocannabinoid system). In 2001, the biological relevance of the fatty acid amide hydrolase (FAAH) [19] in the regulation of anandamide levels was demonstrated using a knockout mice model [20]. As to the 2-AG, growing evidence has assigned to the monoacylglycerol lipase (MAGL) the lead role in the termination of the signalling mediated by this endocannabinoid, despite the fact that no knockout mice model has been reported to date. Here we have reviewed the milestones that punctuated MAGL history, from its discovery to more recent findings in the context of drug discovery and elucidation of its tridimensional structure.

0929-8673/10 $55.00+.00

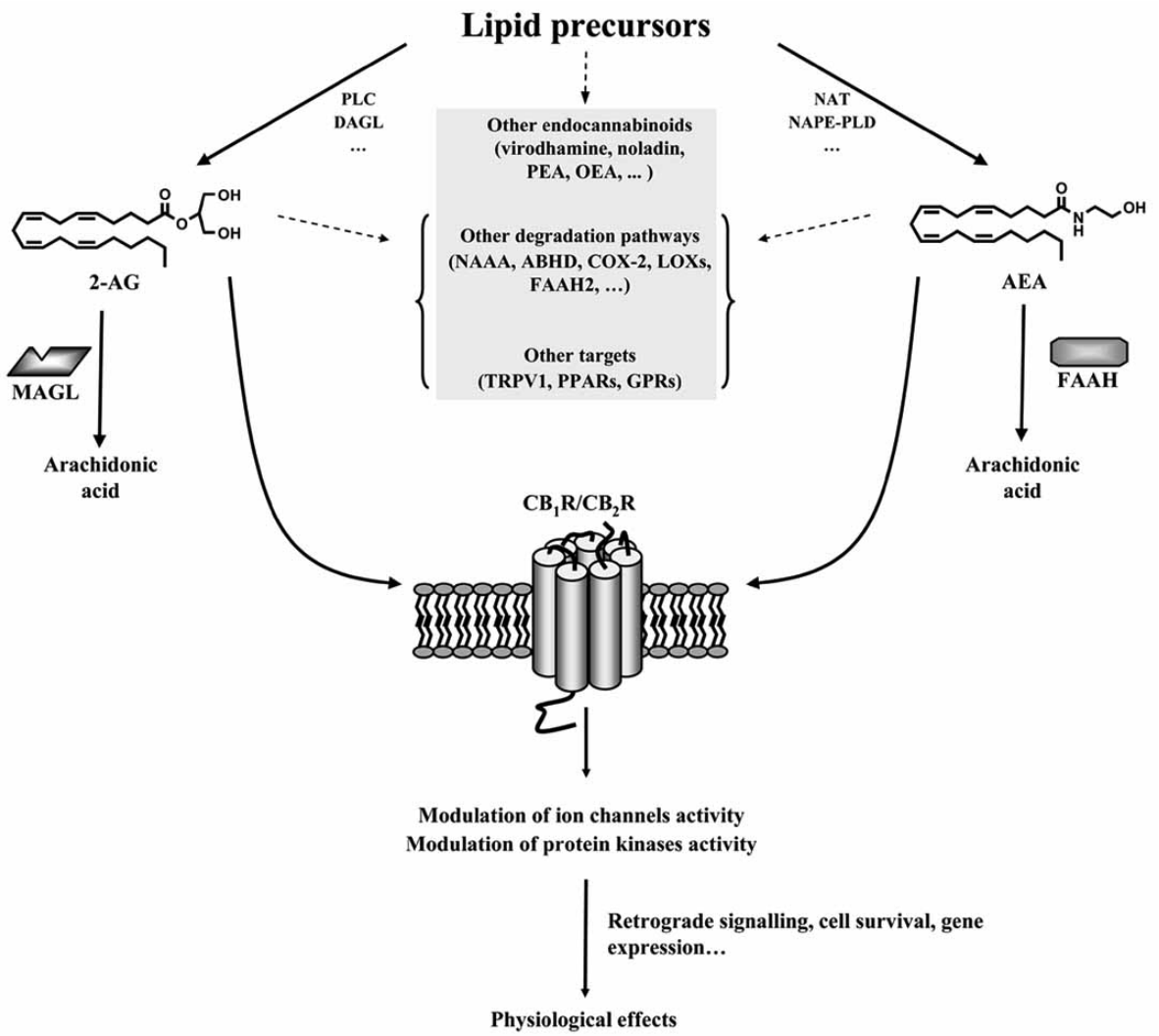

Fig. (1). Overview of the endocannabinoid system. 2-Arachidonoylglycerol and anandamide are synthesized from lipid precursors, and act at CB_1 and CB_2 cannabinoid receptors to generate their physiological effects. Their action is terminated by, respectively, the MAGL and the FAAH. Year after year though, complexity has been added to the system, as new messengers, receptors and metabolic routes have been unravelled. PEA, *N*-palmitoylethanolamine; OEA, *N*-oleoylethanolamine; PLC, phospholipase C; DAGL, diacylglycerol lipase; NAT, N-acyltransferase; NAPE-PLD, *N*-acylphosphatidylethanolamine phospholipase D, NAAA, *N*-acylethanolamine hydrolyzing acid amidase, FAAH2, fatty acid amide hydrolase-2; COX-2, cyclooxygenase-2, LOX, lipoxigenase; PPAR, peroxisome proliferator-activated receptor; ABHD, α/β hydrolase domain-containing proteins.

2. FROM FAT MOBILIZATION TO THE DISCOVERY OF THE MONOACYLGLYCEROL LIPASE

It is not in the context of endocannaboid signalling, but many years before, during the investigation of the pathways that govern lipid metabolism, that the MAGL was brought to light.

Lipid metabolism constitutes a highly regulated process, whose purpose is the maintenance of the subtle balance between fatty acids supply and demand. In the adipocyte, triacylglycerols are packaged into dynamic organelles termed lipid droplets, and are subsequently hydrolysed into fatty acids which are released into blood circulation to face demand [21].

It was only at the beginning of the sixties that several studies demonstrated the presence, in the adipocyte, of two proteins involved in lipolysis and distinct from the lipoprotein lipase [22]. On the one hand, the hormone sensitive lipase (HSL), which was believed to cleave triacylglycerols to monoacylglycerols, and on the other hand a monoacylglycerol selective activity, whose purpose is to complete the hydrolysis process to release the free fatty acid [23].

2.1. The Hormone Sensitive Lipase

Despite the fact that its tridimensional structure is still unsolved, HSL belongs to the α/β hydrolases superfamily (see below). Its catalytic activity involves the common Ser/His/Asp triad, and three important domains have been identified (a dimerization domain, the Ser423/Asp703/His733-containing domain, as well as a polyphosphorylation site-containing domain). Besides numerous posttranslational modification processes, a transcriptional regulation has also

been described. This one occurs through a variability in the exon use as well as from the existence of several transcription starts leading to distinct proteins [24]. Hormone sensitive lipase shows an approximately ten times greater preference for diacylglycerols (DAG) than triacylglycerols (TAG) and monoacylglycerols (MAG) [23,24] and a preference for the primary 1(3)- versus 2-ester position [25].

In 1986, in an elegant immunoprecipitation study, Fredrikson and colleagues established the respective roles of MAGL and HSL [26]. While the action of purified hormone-sensitive lipase on triacylglycerols resulted in an accumulation of 2-monoacylglycerols, selective removal of monoacylglycerol lipase by immunoprecipitation led to a strong decrease in glycerol and fatty acids release from an adipose tissue preparation. The addition of purified monoacylglycerol lipase then allowed the completion of TAG hydrolysis.

Lipolysis, overall, is under tight hormonal control. Among the regulatory processes, the stimulation of either Gi or Gs protein-coupled receptors modulates the activity of the enzymes involved in lipolysis through the control of cAMP levels [21]. For instance, phosphorylation controls HSL through a cAMP-dependent protein kinase (PKA). Activation of β1 and β2 adrenergic receptors stimulates cAMP formation and PKA, leading to an increase in enzyme activity [23,27-30]. The activation of other proteins (including perilipin A) required for the proper action of HSL at the surface of the lipid droplet was later shown to occur simultaneously, both events acting in concert to enhance the lipolysis.

Until quite recently, it was established that fat mobilization inside the adipocyte was under the tight and exclusive dual control of HSL and MAGL, the former initiating the TAG hydrolysis process, and the monoacylglycerol lipase allowing the release of the glycerol and the fatty acid moieties. Things changed when the genetic disruption of hormone sensitive lipase allowed the elucidation of the precise role of the enzyme *in vivo.* Surprisingly, the HSL deficient mice were neither overweight nor obese and even presented a reduced fat mass, while TAG contents remained unchanged and white and brown adipose tissues still exhibited 40% and 100% of triacylglycerol lipase activity, respectively. On the contrary, these mice were characterized by a diacylglycerol accumulation compared to wild-type animals [31-33]. These observations led to the identification of a novel enzyme named adipose tissue triacylglycerol lipase (ATGL) [34].

2.2. The Adipose Tissue Triacylglycerol Lipase

Physiological relevance of ATGL was clearly established when a knockout mice model was reported [35]. Unlike HSL deficient mice, $ATGL^{-/-}$ animals are characterized by a strong fat accumulation, as well as an increase of body weight and fat mass and a reduced lifespan.

Like HSL, ATGL activity is also kept under a tight control by hormonal factors and nutritional status [24]. Whilst TNFα stimulates lipolysis in $HSL^{-/-}$ mice, it reduces ATGL mRNA levels, thus strongly suggesting the occurrence of posttranslational modifications of the enzyme. Activation of the nuclear receptor PPARγ also positively modulates ATGL gene transcription, as rosiglitazone, a PPARγ agonist, enhances mRNA levels and lipolysis [36,37]. Besides transcriptional modification, several proteins associated to the lipid droplets participate in the action of ATGL, and therefore in the regulation of its activity. In particular, CGI-58 (or α/β hydrolase domain-containing 5, ABHD5) is a protein linked to perilipin A at the surface of the lipid droplet. Following β-adrenergic stimulation and the cAMP-dependent PKA activation, perilipin A is phosphorylated. This causes CGI-58 to dissociate from the droplet, and its subsequent interaction with ATGL to activate lipolysis [38-41]. Other lipid-associated proteins in close connection with lipid droplets and ATGL take part into the recruitment of ATGL at the surface of the lipid droplet and the activation of lipolysis. The complex networks regulating lipolysis through the modulation of HSL and ATGL and their physiopathological implications in the context of fat metabolism have been recently discussed in excellent reviews [24,42].

Since the blurred initial picture in the early sixties, when only global lipase activities could be detected - with hardly isolable and unknown actors displaying overlapping substrate selectivities as well as tissular and cellular localization - understanding of the lipolysis process has had a major breakthrough to lead to the current conception (Fig. (**2**)).

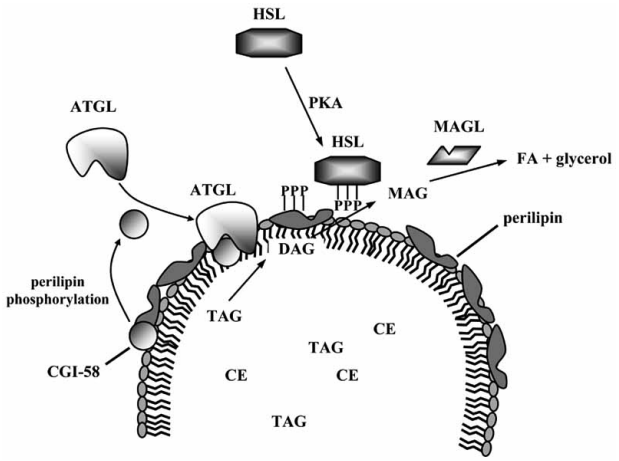

Fig. (2). Schematic representation of the fat mobilization process, taking place at the surface of the lipid droplet in the adipocyte. (CE: cholesteryl ester; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; FA: fatty acid; ATGL: adipose tissue triacylglycerol lipase; HSL: hormone-sensitive lipase; MAGL; monoacylglycerol lipase; PKA: proteine kinase A). Free fatty acids are released following the consecutive action of ATGL, HSL and MAGL. At the surface of the droplet, proteins like perilipin and CGI-58 regulate the translocation and activation of ATGL and HSL.

3. THE MONOACYLGLYCEROL LIPASE

As mentioned above, the first evidence of the presence, in the adipose tissue, of a lipase activity specific for monoacylglycerols and distinct from lipoprotein lipase and HSL was established more than forty years ago [22]. Using different enzyme assays, the authors identified distinct lipase activities in the fat portion of adipose tissue. One of these, co-localized with HSL and other putative lipases, displayed a strong selectivity for mono- versus di- and tri-glycerides.

The biochemical study of this - at the time unknown - lipase activity highlighted a slight preference for 2-monoolein compared to 1-monoolein and an optimum pH of 8.0 [22].

Two years later, the same group reported the partial purification and characterization of the enzyme [43], resulting in the confirmation of the absolute monoacylglycerol preference over diacylglycerols and triacylglycerols and the identification of the first chemical tools to modulate MAGL activity (see below).

In 1976, the first thorough purification of monoacylglycerol lipase was achieved, starting from the adipose tissue from 100 rats [44]. The main advance that allowed the isolation of the MAGL was the use of a detergent to dissociate the enzyme from large lipid-containing aggregates. During this study, it was also shown that detergent and a reducting agent were required to ensure good enzyme stability.

3.1. Cloning of the Monoacylglycerol Lipase

It took many more years before the cloning of the gene encoding MAGL was reported by the same group [45]. Using the same procedure and starting from epididymal fat pads from rats, the authors purified the MAGL, subjected the protein to tryptic digestion, and after sequencing of seven peptides, they amplified a 519 bp PCR product from a mouse adipose tissue cDNA preparation and used the fragment as a probe to screen a mouse adipocyte λgt11 cDNA library. This resulted in the cloning of the whole coding sequence of the mouse MAGL, which was then expressed in COS cells for further characterization.

Since then, a huge amount of MAGL orthologs from many eukaryotics organisms, including human, chimpanzee, dog, cow, rat, chicken, zebrafish, *A. thaliana* and rice have been cloned, often sharing a high degree of identity (Table **1**).

3.2. From the Gene to the Function

Mouse MAGL gene, located on chromosome 6, is composed of seven exons [46]. The cDNA sequence which was initially reported encodes a 303 a.a. protein of 33,218 kDa, a value that is very close to the estimation given by Tornqvist and Belfrage twenty years before (31,800 to 32,900 kDa).

Several bacterial, yeast, and viral proteins were identified as MAGL homologs. The MAGL shares about 50% identity with a poxvirus "hypothetical protein" which was identified the same year [47]. Interestingly, when the gene encoding the latter protein was cloned, it was shown to be highly identical to a human cDNA (HU-K5, actually the MAGL cDNA) and, based on the similarity to an *E.coli* lysophospholipase, the authors suggested that these sequences might encode proteins involved in lysophospholids catabolism.

Other neighbours, sharing between 20% and 25% identity with MAGL, included an esterase from *Pseudomonas putida*, a hypothetical protein from *S.cerevisiae*, proteins from *A.thaliana* and *M.genitalium*, lysophospholipases from *E.coli* and *H.influenzae*, as well as several bacterial haloperoxidases [45]. Alignment of these sequences allowed the identification of the HG dipeptide and the GXSXG motif commonly found in lipases and esterases and which is likely to contain the nucleophilic serine [48]. It also led to propose a putative catalytic triad, constituted by Ser 122, Asp 239 and His 269, whose point mutation confirmed the implication in the catalytic mechanism.

Moreover, comparison with the only close homolog of known tridimensional structure, the bromoperoxidase A2 from *S. aureofaciens*, combined with secondary structure prediction, ranked MAGL among the α/β hydrolase superfamily, whose fold is shared by many lipases, esterases, and haloperoxidases [49].

3.3. Substrate Selectivity

Although the 2-monoolein hydrolase activity in the adipose tissue was initially reported to be two times greater than that for the *sn*-1-isomer [22], a lack of selectivity for both regioisomers was demonstrated later by the use of pure enzyme. The lipase (enzyme classification 3.1.1.23) is unable to cleave either diacylglycerols or triacylglycerols. Moreover, it is inactive on cholesterol esters and prostaglandin-glycerol esters, and despite its known homology with viral and bacterial lysophospholipases, the enzyme does not display any lysophospholipase activity for lysophosphatidylcholine [44,50,51].

Several authors have studied the impact of the length and the degree of unsaturation of the substrate acyl chain, by

Table 1. MAGL Orthologs Found Among Eukaryotes, Ranked Following the Identity in Amino Acids Primary Sequence.

Organism	% identity versus human MAGL	Accession code
Human	100	NP_009214.1
Dog	92.7	XP_533717.2
Cow	85.8	XP_581556.4
Mouse	84.2	NP_035974.1
Rat	83.8	NP_612511.1
Chicken	70.4	XP_414365.2
Zebrafish	56.3	NP_956591.1
Arabidopsis thaliana	32.4	NP_568327.1
Oryza sativa	29.4	NP_001064121.1

measuring either the rate of hydrolysis or the inhibitory potency of the compounds towards the hydrolysis of a known substrate (thus reflecting more the affinity for the enzyme than its capability to act as a substrate). MAGL hydrolyzes efficiently substrates with various number of double bonds (C18:1, C18:2; C20:4) and acyl chain length (from C8:0 to C18:0) [43,52]. However, the degree of unsaturation was reported to have a certain impact, with the MAGL displaying a preference for arachidonoylglycerol compared to palmitoylglycerol and, generally speaking, for unsaturated compared to saturated substrates (C16:0, C20:0) [52-54].

By measuring the inhibitory potency of a series of trifluoromethylketone derivatives on the 2-AG hydrolase activity from a rat cerebellar cytosolic fraction, Ghafouri and colleagues also observed a marked preference for the arachidonoyltrifuoromethylketone (pIC_{50} = 5.54) and oleoyltrifuoromethylketone (pIC_{50} = 5.99) compared to the palmitoyl analogue (pIC_{50} = 5.11) [54].

It is noteworthy to mention that, in several of these studies, data analysis might have been complicated by the fact that protein homogenates are often used as the source of enzyme activity, raising concerns about the exact identity of the enzyme whose activity is measured. The use of recombinant MAGL could therefore be helpful to gain additional information on the enzyme substrate selectivity.

3.4. Tissue Localization

Since its first identification in the adipose tissue, MAGL localization has extended to many other tissues and is now known to constitute an ubiquitously expressed enzyme. In the rat, high levels of MAGL mRNA are found in the adipose tissue, kidney and testis. Other organs include adrenal gland, brain, heart, lung, skeletal muscle, liver, ovary and spleen, in decreasing order of MAGL mRNA abundance [45]. In the brain, MAGL transcript is also present ubiquitously (cortex, hippocampus, cerebellum, thalamus, striatum), despite lower mRNA levels are found in the brainstem and hypothalamus [55]. In the adipocyte, MAGL was found in large lipid-containing aggregates [44]. Besides this, a concomitant distribution in membranes as well as in the cytosol has been reported. First, adenovirus-mediated MAGL expression in HeLa cells led to immunoreactivity and lipase activity localized in both the cytosolic and plasma membranes/particulate fractions of the cells [55]. Second, in the hippocampus, the ultrastructural distribution of the enzyme is homogenous and no compartmental preference is reported [56]. Third, using ABPP-MudPIT (Activity-Based Proteome Profiling with Multidimensional Protein Identification Technology), Blankman and colleagues identified MAGL both in membrane and cytosolic mouse brain proteomes [57].

This dual MAGL cellular localization (Table **2**) may either suggest the intrinsic amphitropic nature of MAGL or reflect putative posttranscriptional/posttranslational events regulating MAGL expression and activity.

3.5. Regulation of MAGL Activity

Unlike the hormone-sensitive and adipose tissue triacylglycerol lipase, which are strongly controlled by hormonal factors, little is known about the regulation of MAGL, either at the gene or posttranslational levels. In view of the high abundance of monoacylglycerol lipase activity in the adipocyte and the apparent lack of regulation by hormonal factors, Vaughan and colleagues suggested that HSL and not MAGL might constitute the rate-limiting step in the fat mobilization process [22]. Nevertheless, more recent observations support the existence of several kinds of regulation of MAGL.

First, a western blot analysis of MAGL tissular expression in different tissues has revealed the existence of MAGLs of distinct molecular weights [46]. In the mouse, whereas MAGL from adipose tissue, liver, heart, lung, stomach, kidney, spleen, kidney and adrenal gland migrates with a molecular weight of about 33 kDa, the enzymes from the

Table 2. Evidence for the Amphitropic Localization of the MAGL. The enzyme was observed in both the membrane and cytosolic fraction of neurons, or after overexpression in eukaryotic cells. Besides this, the enzyme was also purified from adipocytes lipid aggregates. (ABPP-MudPIT: Activity-based Proteome Profiling with Multidimensional Protein Identification Technology)

Enzyme source	Evidence	Localization	References
Expression in HeLa cells	Activity assay/western blot, before and after immunodepletion	Cytosolic fraction	[58]
Expression in HeLa cells	Activity assay Immunohistochemistry	Cytosol and plasma membrane (particulate fraction)	[55]
Expression in COS7 cells	ABPP	Membrane and cytosol	[57]
Rat cerebellum	Activity assay	Membrane fraction	[59]
Rat cerebellum	Activity assay	Cytosolic and membrane fractions	[60]
Mouse brain	Activity assay/ABPP-MudPIT	Membrane and cytosol	[57,61,62]
Rat brain	Western blot	Cytosolic fraction	[55,58]
Rat brain	Activity assay/Western blot, before and after immunodepletion	Cytosolic fraction	[58]
Mouse adipocyte tissue	Isolation of the monoacylglycerol lipase activity	Lipid aggregates	[43] [44] [45]

brain, testis and skeletal muscle seem to constitute other slightly different species. In the brain, and testis, the classical band is accompanied by another one, with a ~2 kDa higher molecular weight. In the muscle, a single and much larger protein was detected (about 40 kDa), whilst in the testis, MAGL either migrates as a single slightly smaller protein (~30 kDa) or constitutes two species differing by 2 kDa, similarly as for the brain enzyme [46,55,57,62]. Whether such entities arise from the use of different in frame start codons, from alternative splice variants, or from posttranslational modifications of the enzyme is still unknown. Nevertheless, at the gene level, several possibilities exist. First, two isoforms are reported in the GenBank (long, isoform 1, accession code NP_001003794; short, isoform 2, accession code NP_009214) (An alignment is shown in Fig. (**3**)). Consistently, Karlsson and colleagues reported the identification of divergent 5' leader sequences for MAGL cDNA. The sequences differ 21 bp upstream from the predicted translation start previously reported by the same group [45] and the longest form contains two additional in-frame start codons compared with the known protein. The use of these ATG codons would result, at best, in a ~2 kDa increment, and could thus potentially explain the additional band observed in the brain. However, the ~40 kDa and ~30 kDa proteins observed in muscle or testis, respectively, could not arise from these alternative mRNA transcripts.

Moreover, two novel splice variants of the short and long forms have been recently introduced in the GenBank (accession code ACD37712 and BAH14267, unpublished results), and correspond to a deletion of the exon 5. Most importantly, this deletion results in a 30 a.a. deletion which occurs within the so-called cap domain (see below and Fig. (**3**)). In view of the crucial functions played by this region for substrate recognition and selectivity, this alternative splicing is likely to have profound impacts on MAGL function, in terms of cellular localization or activity, for instance.

Another kind of modulation of MAGL activity could involve a regulation of the transcription of the gene. Recently, transcriptional upregulation of MAGL by Peroxisome Proliferator Activated Receptor α (PPARα) has been reported [63]. PPARs are nuclear receptors that, upon heterodimer formation with retinoid X receptor (RXR), bind to target gene promoters to activate transcription. Further, endogenous ligands or drugs can bind PPARs to modulate its activity. In the above-mentioned study, the authors screened mouse liver genes for those whose transcription is altered following PPARα activation. Those involved in lipid metabolism were then studied further. It could be demonstrated that the PPARα agonist Wy14643 led to a 6-fold increase in MAGL mRNA levels. The specificity of this effect was confirmed with the use of a PPARα$^{-/-}$ mouse model and in cultured hepatocytes. This observation makes sense, given the

```
Human, short isoform       1   ---------- MPEESSPRRT PQSIPYQDLP HLVNADGQYL FCRYWKPTGT
Human, long isoform        1   METGPEDPSS MPEESSPRRT PQSIPYQDLP HLVNADGQYL FCRYWKPTGT
Splice variant (short)     1   ---------- MPEESSPRRT PQSIPYQDLP HLVNADGQYL FCRYWKPTGT
Splice variant (long)      1   METGPEDPSS MPEESSPRRT PQSIPYQDLP HLVNADGQYL FCRYWKPTGT

Human, short isoform      41   PKALIFVSHG AGEHSGRYEE LARMLMGLDL LVFAHDHVGH GQSEGERMVV
Human, long isoform       51   PKALIFVSHG AGEHSGRYEE LARMLMGLDL LVFAHDHVGH GQSEGERMVV
Splice variant (short)    41   PKALIFVSHG AGEHSGRYEE LARMLMGLDL LVFAHDHVGH GQSEGERMVV
Splice variant (long)     51   PKALIFVSHG AGEHSGRYEE LARMLMGLDL LVFAHDHVGH GQSEGERMVV

Human, short isoform      91   SDFHVFVRDV LQHVDSMQKD YPGLPVFLLG HSMGGAIAIL TAAERPGHFA
Human, long isoform      101   SDFHVFVRDV LQHVDSMQKD YPGLPVFLLG HSMGGAIAIL TAAERPGHFA
Splice variant (short)    91   SDFHVFVRDV LQHVDSMQKD YPGLPVFLLG HSMGGAIAIL TAAERPGHFA
Splice variant (long)    101   SDFHVFVRDV LQHVDSMQKD YPGLPVFLLG HSMGGAIAIL TAAERPGHFA

Human, short isoform     141   GMVLISPLVL ANPESATTFK VLAAKVLNLV LPNLSLGPID SSVLSRNKTE
Human, long isoform      151   GMVLISPLVL ANPESATTFK VLAAKVLNLV LPNLSLGPID SSVLSRNKTE
Splice variant (short)   141   GMVLISPLVL ANPESATTFK ---------- ---------- ----------
Splice variant (long)    151   GMVLISPLVL ANPESATTFK ---------- ---------- ----------

Human, short isoform     191   VDIYNSDPLI CRAGLKVCFG IQLLNAVSRV ERALPKLTVP FLLLQGSADR
Human, long isoform      201   VDIYNSDPLI CRAGLKVCFG IQLLNAVSRV ERALPKLTVP FLLLQGSADR
Splice variant (short)   161   VDIYNSDPLI CRAGLKVCFG IQLLNAVSRV ERALPKLTVP FLLLQGSADR
Splice variant (long)    171   VDIYNSDPLI CRAGLKVCFG IQLLNAVSRV ERALPKLTVP FLLLQGSADR

Human, short isoform     241   LCDSKGAYLL MELAKSQDKT LKIYEGAYHV LHKELPEVTN SVFHEINMWV
Human, long isoform      251   LCDSKGAYLL MELAKSQDKT LKIYEGAYHV LHKELPEVTN SVFHEINMWV
Splice variant (short)   211   LCDSKGAYLL MELAKSQDKT LKIYEGAYHV LHKELPEVTN SVFHEINMWV
Splice variant (long)    221   LCDSKGAYLL MELAKSQDKT LKIYEGAYHV LHKELPEVTN SVFHEINMWV

Human, short isoform     291   SQRTATAGTA SPP
Human, long isoform      301   SQRTATAGTA SPP
Splice variant (short)   261   SQRTATAGTA SPP
Splice variant (long)    271   SQRTATAGTA SPP
```

Fig. (3). Alignment of the amino acids sequences corresponding to MAGL splice variants with the known sequence of the enzyme (short and long isoforms). The portion of the sequence corresponding to the cap region is underlined.

well documented role of the endocannabinoid system in the energy balance and metabolism [64]. For instance, the CB_1 inverse agonist rimonabant (SR141716A) has been marketed by Sanofi Aventis group to improve the metabolic profile associated with obesity. In addition to this, it is to note that PPARα constitutes the molecular targets for the well known fibrates.

As far as we know, no evidence of phosphorylation or other modifications of MAGL has been reported to date. Nevertheless, while little is known about the putative post-transcriptional or posttranslational modifications encompassing MAGL expression, there is a possibility that such processes might act in unison to regulate MAGL activity, by generating enzymes of different intrinsic activity or subcellular localization. Such species would meet the specific needs of the cell in a particular tissue or physiological situation.

3.6. On the *In Vivo* Relevance of MAGL: From the Fat Mobilization to the Regulation of Endocannabinoid Signalling

MAGL completes the hydrolysis of triacylglycerols in the adipocyte. It behaves as a selective 2-monoacylglycerol hydrolase, devoid of activity on other lipids. This observation turned out to be of utmost importance in the middle of the nineties, when the endocannabinoid system was brought to light. Indeed, besides its original role in the mobilization of fat in the adipocyte, which led to its discovery in the early sixties, the discovery of 2-arachidonoylglycerol as a key messenger of the endocannabinoid system marked a turning point in the study of MAGL.

Despite the lack of adequate pharmacological tools for MAGL - no deficient mice model is available yet and selective, potent inhibitors have been lacking for a long time - several studies have highlighted the physiological relevance of MAGL in 2-AG degradation:

First, in HeLa cells and rat cortical neurons, adenovirus-mediated MAGL expression caused an increase in 2-AG hydrolase activity [55]. In these cells, interfering RNA resulted in a decrease of MAGL lipase activity and enhanced 2-AG levels [58].

Second, MAGL lipase immunodepletion in rat brain soluble fraction leads to a 50% decrease in 2-AG hydrolase activity. Noteworthy, the remaining (and not FAAH-dependent) hydrolytic activity suggests the probable existence of additional enzymes regulating 2-AG levels in the brain [58].

Blankman and colleagues also elegantly contributed to the elucidation of the enzymatic pathways governing 2-AG catabolism [57]. Using the ABPP-MudPIT (Activity-Based Proteome Profiling coupled with Multidimensional Protein Identification Technology), mouse brain was mapped for the enzymes involved in 2-AG degradation. This allowed to assign about 85% of the 2-AG catabolism to MAGL and to limit the FAAH involvement in the degradation of the endocannabinoid to ~1%. Consistently, previous studies had shown that, despite FAAH displaying a high activity towards 2-AG *in vitro*, administration of the selective inhibitor URB597 as well as disruption of FAAH gene ($FAAH^{-/-}$ mice) is not, in most cases, associated with enhanced 2-AG levels [65-67]. As well as these findings, the study of Blankman allowed the identification of two additional hydrolases, the α/β hydrolase domain-containing 6 and 12 (ABHD6 and ABHD12), which were previously uncharacterized and are together responsible for ~13 % of 2-AG degradation.

Finally, treatment of mice with JZL184, one of the most potent and selective MAGL inhibitor, resulted in a ~90% inhibition of 2-AG hydrolysis and a substantial increase of 2-AG brain levels (2-fold to 8-fold, following the dose which was administered) [62]. It also enhanced, to a lower extent however, oleoylglycerol and palmitoylglycerol concentrations. Other tissues were also affected by JZL184 treatment. In kidneys, spleen, heart, brown and white adipose tissue and lungs, MAGL inhibition led to the accumulation, to varying extents, of the three monoacylglycerols tested. Notably, 2-AG levels in white adipose tissue and lung did not increase, unlike those of C16:0 and C18:1 monoacylglycerols. Moreover, no statistically significant change in the monoacylglycerols levels was observed in the testis. In the testis and adipose tissue, about 50-60% of the 2-AG hydrolyzing activity remained after JZL184 treatment. Whether this results from the presence of additional lipase(s), like hormone-sensitive lipase in the adipose tissue, or from the limited JZL184 ability to completely inhibit MAGL in these tissues is still questionable. Another possibility is that the differences between the alternative MAGL species (splice variants, posttranslational modifications) might have an impact on how well the protein is accessible to - or inhibited by - JZL184.

Overall, evidence tends to demonstrate the crucial involvement of MAGL in the regulation of 2-AG pools. It is likely that the availability of $MAGL^{-/-}$ mice model and the emergence of more potent, and above all more selective, inhibitors will provide even more precise answers to the question of the MAGL biological function.

3.7. MAGL and the Endocannabinoid Retrograde Signalling

One of the essential functions of CB receptors and endocannabinoids is to be involved in synaptic plasticity, as they modulate the signaling mediated by other transmitters. Depolarization of the postsynaptic neuron, as well as the activation of Gq protein-coupled receptors, results in the activation of endocannabinoid biosynthesis pathways in the postsynaptic neuron (Fig. (**4**)) [13]. The newly synthesized endocannabinoid travels backward, crossing the synaptic space to bind presynaptic CB_1 receptors, inhibiting calcium influx and decreasing the probability of presynaptic vesicle release. Overall, this chain of events leads to a temporary or more persistent dimming of inhibitory or excitatory currents at GABAergic and glutamatergic synapsis, respectively.

The subtlety of this modulation is further strengthened by the intervention of endocannabinoid degradating pathways, and indeed growing evidence has emerged to assign a key role to MAGL in the endocannabinoid-mediated retrograde signalling.

- Immunohistochemistry and light microscopy studies have allowed the study of subcellular and ultrastructural localization of MAGL and FAAH in several

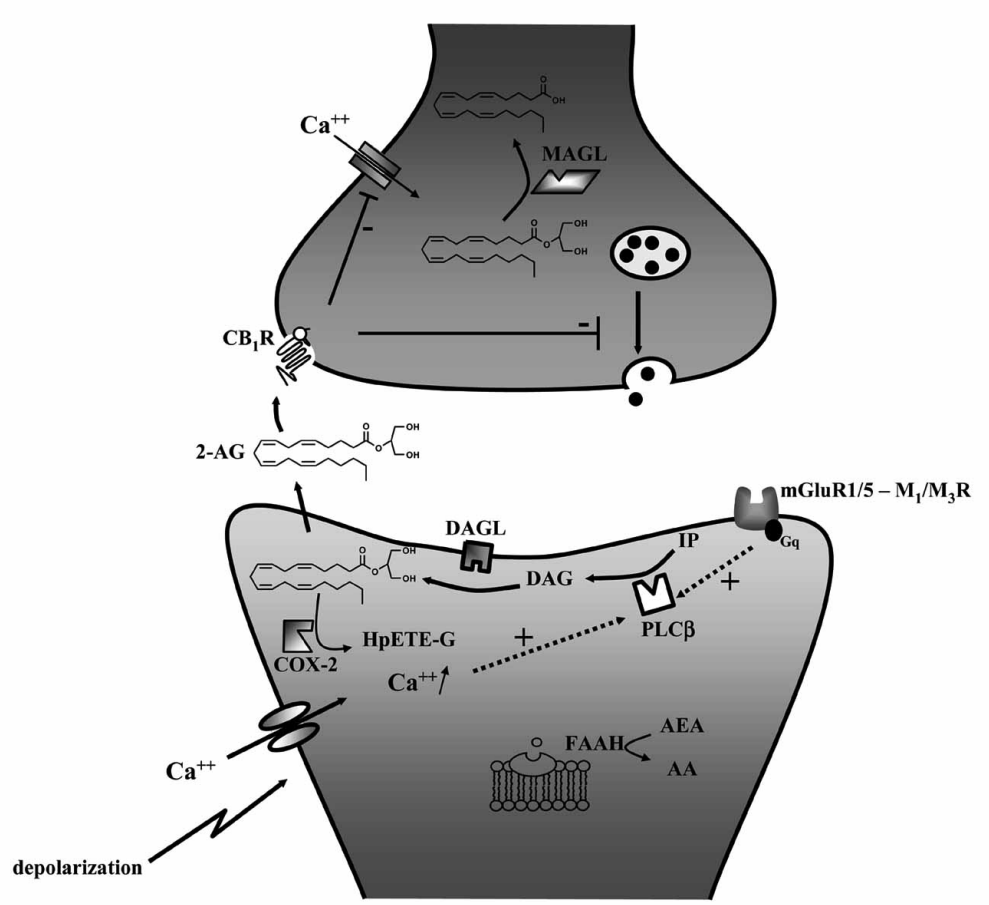

Fig. (4). The MAGL modulates the 2-AG-dependent retrograde signaling in the presynaptic neuron. Following the on demand synthesis of 2-AG, the endocannabinoid stimulates presynaptic CB_1 receptor, thus decreasing the release of other neurotransmitters. The MAGL, which is exclusively located in the presynaptic neuron, modulates this inhibitory signal. In addition, in the postsynaptic neuron, the COX-2 appears to regulate part of the postsynaptic pool of 2-AG. On the contrary, the FAAH, which is anchored to the membranes of intracellular compartments, does not seem to be involved in the control of 2-AG, despite the fact that it hydrolyses this substrate very efficiently *in vitro*.

brain areas. This sheds light on the spatial organization of the endocannabinoid actors at the synaptic level, compatible with the intrinsic function of the system in neural transmission [56]. In the hippocampus, the cerebellum and the amygadala, MAGL and FAAH co-localize with CB_1 receptor and display a complementary distribution. MAGL is exclusively found in the presynaptic axon terminals of inhibitory and excitatory interneurons, along with CB_1 receptors. On the contrary, FAAH is associated with dendrites and neuron somata but is absent from axon terminals. Interestingly, at the ultrastructural level, FAAH is associated with the cytoplasmic surface of smooth endoplasmic reticulum cisternae and cytoplasmic surface of mitochondrial outer membranes, but locates more rarely on the cell membrane, whilst MAGL shows a much more diffuse pattern, with no compartmental preference.

- MAGL overexpression in HeLa cells counteracts the synaptic 2-AG (but not AEA) accumulation resulting from *N*-methyl-D-asparate and acetylcholine receptors activation [68], which had been previously demonstrated to enhance 2-AG and anandamide production, leading to the retrograde inhibitory signal effects observed in the the hippocampal and the cortex [55,69].

- Despite the paucity of MAGL inhibitors available to date, several studies using compounds interfering with MAGL (with more or less selectivity) have also allowed a better understanding of the role played by MAGL in the hippocampal depolarization-induced suppression of inhibition (DSI). First, in CA1 pyramidal cells, URB602, a weak inhibitor of both MAGL and FAAH [60,70], increases duration of DSI, unlike the selective FAAH inhibitor URB597 [71,72]. Second, in other studies on hippocampal neurons, MAFP and ATFMK, two non selective FAAH/MAGL inhibitors, also ***i.*** decrease inhibitory and excitatory post-synaptic currents (IPSC, EPSC) at basal state and ***ii.*** extend the duration of DSI and its excitatory counterparts (depolarization-induced suppression of excitation, DSE), and the inhibitory signal induced by exogenous 2-AG [73]. Once again, in this study, URB597 failed to change duration of DSI, as reported previously by other teams, whilst the selective COX-2 inhibitor meloxicam prolonged the dura-

tion of DSI but not the inhibitory action of exogenously applied 2-AG [70,72]. Third, the recent development of the selective MAGL inhibitor JZL184 (see below) has provided a priceless tool for determining the nature of the pathways involved in the endocannabinoid-mediated retrograde signalling. By demonstrating that JZL184 extends the duration of DSE in cerebellar neurons and DSE/DSI in hyppocampal pyramidal neurons, Straiker, Pan and their colleagues recently provided additional and convincing evidence for the predominance of the 2-AG/MAGL pathway over that involving AEA/FAAH in the DSI/DSE process [74,75].

Thus, the CB_1/FAAH/MAGL tissular co-localization, the spacial segregation of MAGL and FAAH, as well as the disruption of MAGL, FAAH and COX-2 activities by pharmacological tools have shed light on the functioning of the endocannabinoid system within the synapse (Fig. (**4**)). When activated by a strong depolarization or GPCR activation, postsynaptic neurons synthesize and accumulate 2-AG and/or AEA. The endocannabinoid diffuses locally towards the presynaptic terminal where it activates cannabinoid receptors, causing an inhibition of Ca^{++} influx and a decrease in neurotransmitter release. The identity of the endocannabinoid which is involved in this process (2-AG versus anandamide) is, however, still a matter of debate. In the short-term form of synaptic plasticity (depolarization-induced form or receptor-driven endocannabinoid release), it is now believed that presynaptic MAGL, but not postsynaptic FAAH, terminates the retrograde inhibitory signal. This observation thus involves 2-AG as mediator. Postsynaptic COX-2 could also participate in the regulation of endocannabinoid accumulation.

In the longer form of plasticity (LTD, long term depression), the production of either anandamide or 2-AG is involved, depending on the areas of the brain. LTD in the cerebellum and the hippocampus could be mediated by 2-AG, whereas it might involve anandamide in the striatum and amygdala [73,76-78].

3.8. Recent Findings on MAGL and its Implication in Cancer Pathogenesis

In the previous paragraphs, the emphasis was on the functioning of the endocannabinoid system in the retrograde signaling within the synapse. This essential function takes place in several regions in the brain including the hypothalamus, cerebellum, hippocampus, striatum, neocortex, amygdala, striatum and nucleus accumbens, where its purpose seems to be its ability to provide protective/homeostatic responses to stresses and to regulate affective, cognitive or motivational processes [10].

Beside the central nervous system however, the endocannabinoid system is also well prominent in the periphery, where it plays an essential role in the control of a wide range of biological functions such as the control of nociception, metabolism, reproduction, cardiovascular and gastrointestinal function and bone formation [14].

Recently, the implication of the MAGL in cancer pathogenesis has been demonstrated, raising new hopes for the development of original anticancer therapeutics based on MAGL inhibition [79]. In their study, Nomura and colleagues found elevated MAGL expression levels in aggressive versus nonaggressive human cancer cell lines. This was accompagnied by a profound decrease in the concentration of several monoacylglycerols, as well as an increase in the corresponding fatty acids. On the one hand, MAGL overexpression increased the aggressiveness of non aggressive cancer cell lines. On the other hand, genetic knockdown of MAGL expression by interfering RNAs, as well as inhibition of the enzyme activity with an JZL184, a selective inhibitor (see below), affected the migration, invasion, cell survival and tumor growth in aggressive cancers. Importantly, exogenous fatty acids restored the loss of pathogenicity. Overall, it was shown that, in cancer cells, a panel of fatty acids and other lipids are produced following the stimulation of MAGL activity to promote cell proliferation and cancer malignancy.

3.9. From a Pharmacological Tool to a Drug Targeting MAGL

During the last decade, the sudden awareness of the implication of endocannabinoid system in such a pleiotropy of functions prompted the medicinal chemist to develop tools in order to explore the pathways governing endocannabinoids degradation, as well as to validate MAGL as a therapeutic target. Such a quest has been made necessary especially since MAGL knockout mice model is still unavailable to date.

3.9.1. Targeting Cysteines

A. First inhibitors of MAGL were described forty years ago and comprise several sulfhydryl-reacting compounds. Such agents include *p*-chloromercuribenzoate (pCMB), mercurichloride ($HgCl_2$) and *N*-ethylmaleimide (NEM) [43,44]. Based on the known inhibitory potential of NEM and a three dimensional model of MAGL showing the presence of two cysteines in the active site of MAGL (Cys208 and Cys242), Saario and colleagues synthesized a series of maleimide derivatives, whose mode of inhibition involves a Michaelis addition on one or several sulfhydryl residue(s) (Fig. (**5**)) [59,80].

This resulted in the development of *N*-arachidonylmaleimide (NAM), the most potent compound using this scaffold, with an IC_{50} value of 0.14 μM on rat cerebellar membranes [59] (Fig. (**6**)). Noteworthy, other groups subsequently reported higher IC_{50} values for this compound [57,80]. Our group also reported the synthesis of a series of maleimides [81]. For instance, 1-biphenyl-4-ylmethylmaleimide inhibits pure human MAGL with an IC_{50} of 790 nM, whilst being 2 orders of magnitude less potent on the FAAH.

Using ABPP, Blankman and colleagues confirmed the relative NAM selectivity for MAGL, although a partial inhibition of FAAH and ABHD12 was also observed at higher dose. Hence, the compound was used as a pharmacological tool to unveil the consequences of a disruption of MAGL activity and to elu-

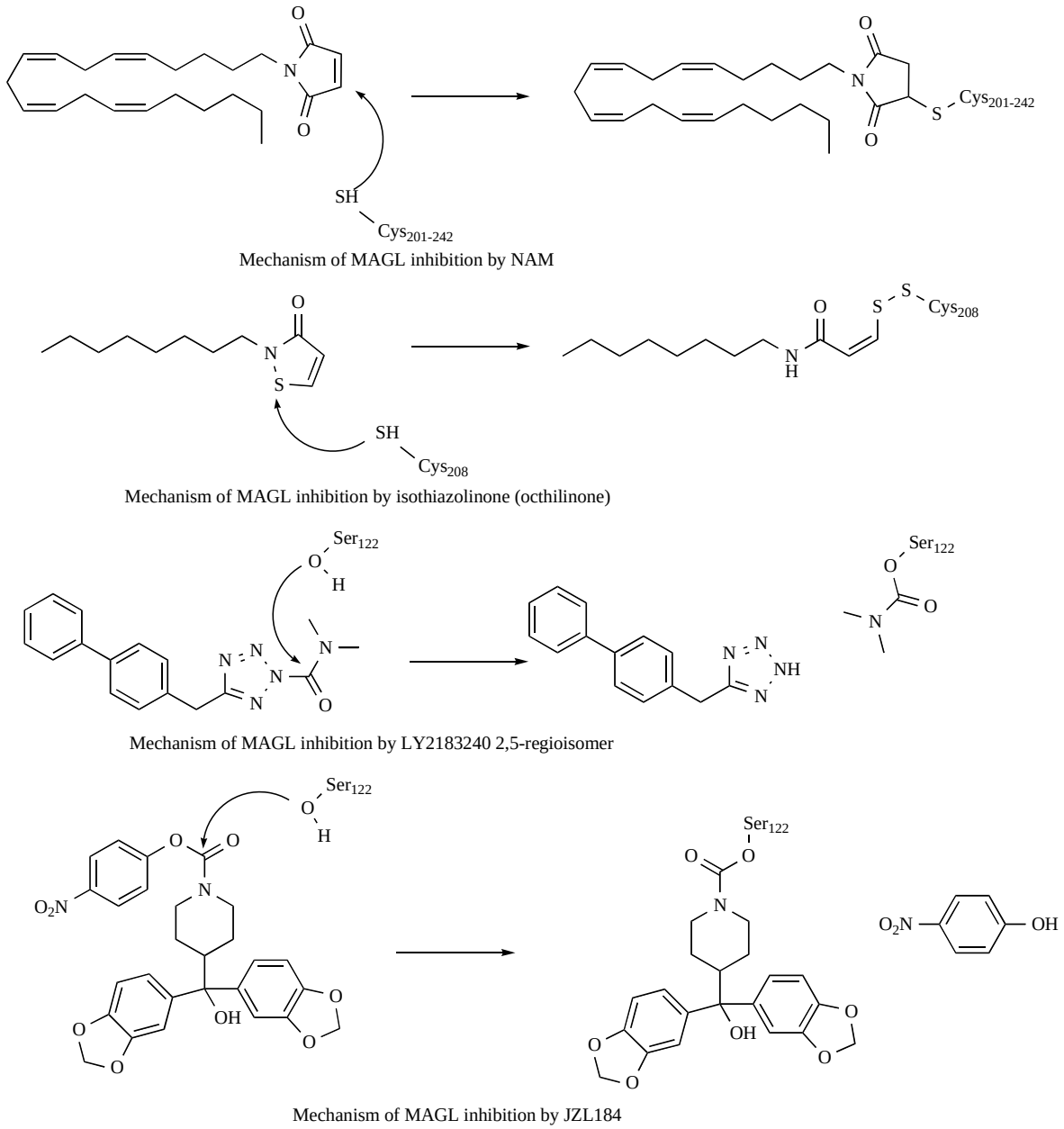

Fig. (5). Mechanism of action of several of the most representative MAGL covalent inhibitors.

cidate the respective effects of 2-AG versus AEA [82]. However, given the fact that *in vivo*, NAM is likely to result in the covalent modification of a huge amount of proteins – not necessarily in the active site – there is little chance that this compound would be a good candidate for drug development.

B. Disulfiram, a well known aldehyde dehydrogenase inhibitor used since decades to treat alcoholism, was reported to inhibit human MAGL with a micromolar affinity (Fig. (**6**)) [83]. Recently, Kapanda and colleagues also reported the development of a series of MAGL inhibitors based on this template. The most potent representative, bis(4-methyl-1-piperazinylthiocarbonyl)disulfide, displayed an IC_{50} of 110 nM on the pure enzyme, and a more than 1000 selectivity ratio for MAGL compared to FAAH. Using dithiothreitol and site-directed mutagenesis, the inhibition was shown to occur through an interaction with the Cys242 and Cys208, inside MAGL catalytic site [84].

C. Very recently, while investigating a series of sulfhydryl-reactive agents, King and colleagues have reported the ability of the 2-octyl-isothiazol-3-one to inhibit the rat MAGL (octhilinone, IC_{50} = 88 nM on pure rat MAGL) (Fig. (**6**)). The compound inhibits the enzyme through a partially reversible mechanism. According to site-directed mutagenesis experiments, the authors suggested the formation of a disulfide adduct with the Cys208 (Fig. (**5**)). In addition, substituting the *n*-octyl substituent with an oleoyl group increased the inhibitory potency (2-octadec-9-enyl-isothiazol-3-one, IC_{50} = 43 nM, on rat MAGL) [85].

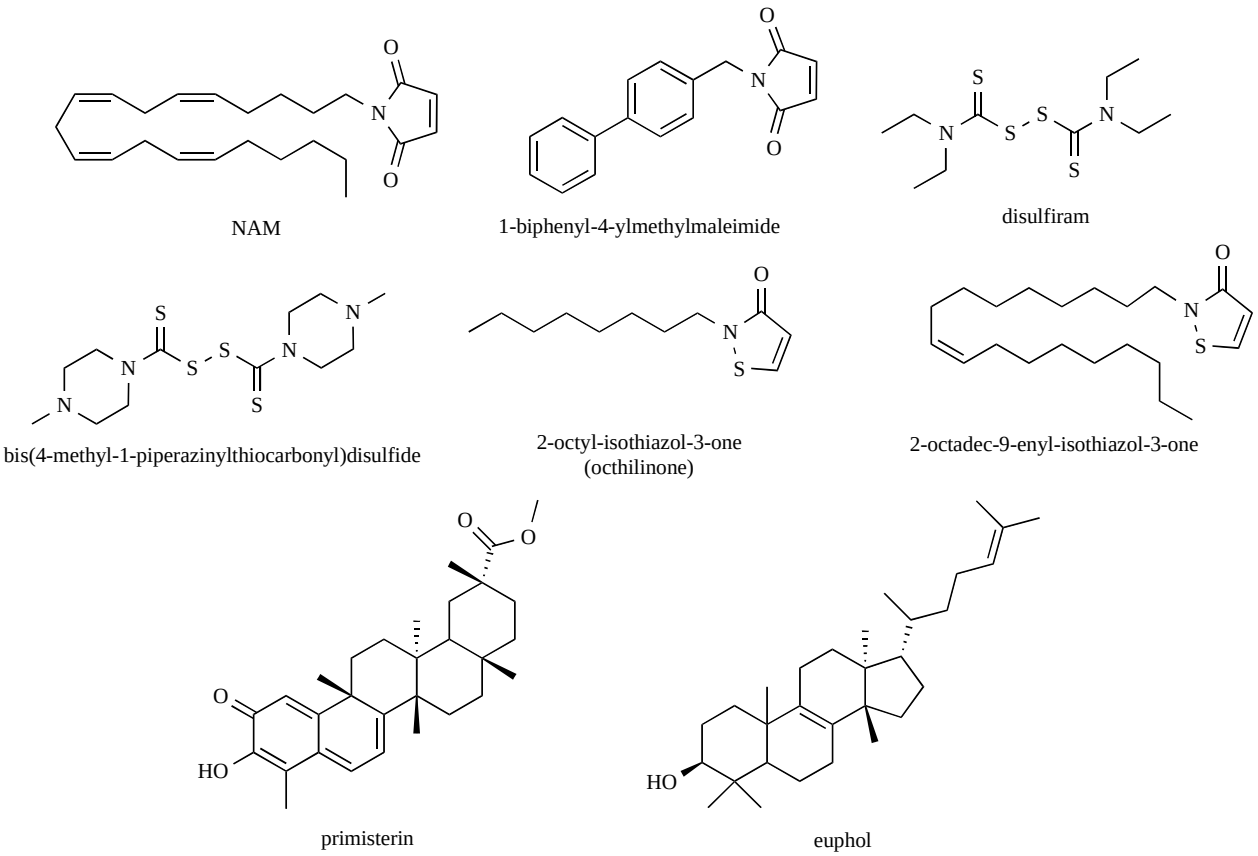

Fig. (6). Most representative examples of inhibitors targeting one or several cysteine residue(s) inside the catalytic site of the MAGL.

Besides this, the same authors identified pristimerin and euphol, two natural terpenoids, as inhibitors of rat MAGL (IC_{50} on rat MAGL: primisterin, 93 nM; euphol, 315 nM). Most interestingly, these compounds act through a reversible mechanism, by interacting with either Cys201 or Cys208 [86].

3.9.2. Targeting the Nucleophilic Serine

A. First characterization of MAGL identified diisopropylfluorophosphate as MAGL inhibitor [43,44]. Other compounds include trifluoromethylketones (arachidonoyltrifluoromethylketone, ATFMK; oleoyltrifluoromethylketone; palmitoyltrifluoromethylketone) and the irreversible inhibitor methylarachidonylfluorophosphonate (MAFP) [54,83] (Fig. (**7**)). Despite the fact that the voluminous and lipophilic arachidonyl chain would be likely to provide MAFP and ATFMK an improved selectivity profile versus proteins belonging to other systems, these compounds also display several off-targets. MAFP was first reported as a phospholipase A2 inhibitor and inhibits FAAH and MAGL with similar potencies, while ATFMK acts on MAGL, FAAH and CB_1 cannabinoid receptor.

B. A series of trifluoromethylketones with a β-thioether moiety were developed by Nithipathikom [87,88]. A subset of these compounds, among which octylthiotrifluoropropan-2-one (OTFP), increased 2-AG levels in two prostate cancer cell lines, revealing the anti-invasive effect of 2-AG. However, their inhibitory potency was only tested in crude cytosolic and membrane homogenates, and it is likely that these compounds unselectively target other esterases and probably the FAAH, since it is known that trifluoromethylketone scaffold constitutes a good template for FAAH inhibition [89].

C. Ely-Lilly carbamoyl tetrazole LY2183240 was first described as an inhibitor of anandamide transporter (IC_{50}^{uptake} = 15 nM) [90] (Fig. (**7**)). However, it has been subsequently reported that this property could result from its ability to potently and irreversibly inhibit FAAH as an off-target (IC_{50}^{FAAH} = 21 nM). LY2183240 was also shown to inhibit other lipases and carboxylases, as assessed by competitive ABPP *in vitro* and *in vivo* (IC_{50}^{MAGL} = 5.3 nM ; IC_{50}^{Abh6} = 0.09 nM ; $IC_{50}^{KIAA1363}$ = 8.2 nM) [91,92]. Indeed, as pointed out by Ortar and colleagues, the compound studied by Alexander and Cravatt likely represents a mixture of the 1,5- and 2,5-regioisomers of LY2183240 [93]. The authors thus synthesized and purified both isomers and other tetrazole derivatives, and measured their inhibitory potency on the MAGL and the FAAH, as well as on the anandamide transport process. On the one hand, the 1,5-regioisomer only slighly inhibits MAGL, whilst being a much more potent inhibitor of anandamide degradation and transport (IC_{50}^{FAAH} = 2.1 nM ; IC_{50}^{uptake} = 15 nM ; IC_{50}^{MAGL} = 8100 nM). On the other hand, the 2,5-

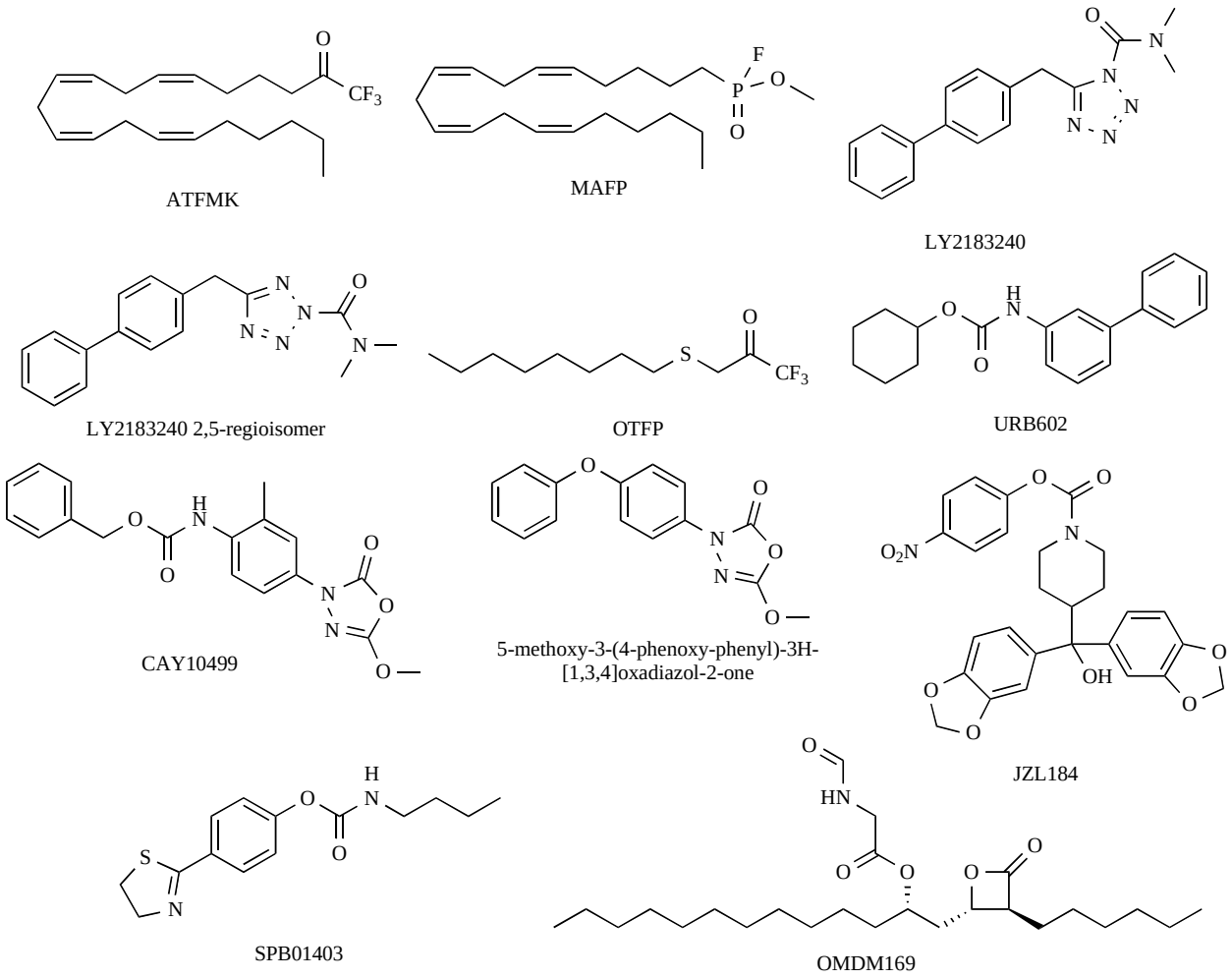

Fig. (7). Several MAGL inhibitors targeting the nuclephilic serine (Ser122)

isomer interacts with a much higher affinity with MAGL, but unfortunately lacks selectivity versus FAAH (IC_{50}^{FAAH} = 33 nM ; IC_{50}^{uptake} = 998 nM ; IC_{50}^{MAGL} = 20 nM). In a mass spectrometry analysis, the binding mode of the 2,5-regioisomer to recombinant human MAGL was investigated. Inhibition occurs through the attack of the Ser122 hydroxyl group, which leads to the formation of a carbamoylation product containing the inhibitor dimethylamino group, as demonstrated by the +71 mass increment of the tryptic fragment containing this residue. The biphenylmethyl tetrazole moiety thus constitutes the leaving group during this reaction [80].

D. Sanofi-Aventis group recently reported the development of a series of triazolopyridine carboxamides and triazolopyrimidine carboxamides derivatives that show a dual or selective inhibitory activity versus 2-OG and AEA hydrolase activity in mouse brain homogenates [94-96] (Fig. (**8**)). Despite the fact that only a subset of data are reported in these patents, these compounds seem to display interesting inhibitory potencies towards either MAGL or FAAH.

E. Based on the discovery of the inhibitory potency of tetrahydrolipstatin (THL), a lipase inhibitor, on 2-AG hydrolase activity in brain [57], Di Marzo's team reported the synthesis of a series of THL-derivatives, which led to the development of the micromolar inhibitor OMDM169 [97] (Fig. (**7**)). Unlike several of the above-mentioned compounds, OMDM169 displays a good selectivity for 2-AG versus AEA degradation (IC_{50} = 0.13-0.34 μM on 2-AG hydrolysis in rat brain and rat cerebellum preparations; IC_{50} = 3.0 μM on rat FAAH). Interestingly, OMDM169 is reversible and its inhibitory potency varies depending on the enzyme source and the species, as the compound inactivates 2-AG hydrolysis about ten times more strongly in rat than in mouse tissues (IC_{50} = 0.92 to 7.53 μM in mouse). Considering the multiple pathways that inactivate 2-AG *in vitro*, the authors also measured the ability of OMDM169 to inhibit pure recombinant human MAGL (IC_{50}^{hMAGL}= 0.89 μM). Despite the fact that the whole spectrum of off-targets has not yet been fully investigated, several enzymes may already be pointed out. These are the diacylglycerol lipase, with a potency similar to that on FAAH (IC_{50}^{DAGL}= 2.8 μM), the human pancreatic lipase (IC_{50}^{HPL}= 0.6 μM) and hepatic triacylglycerol lipase. As this compound initially originates from THL, a well known hormone sensitive lipase inhibi-

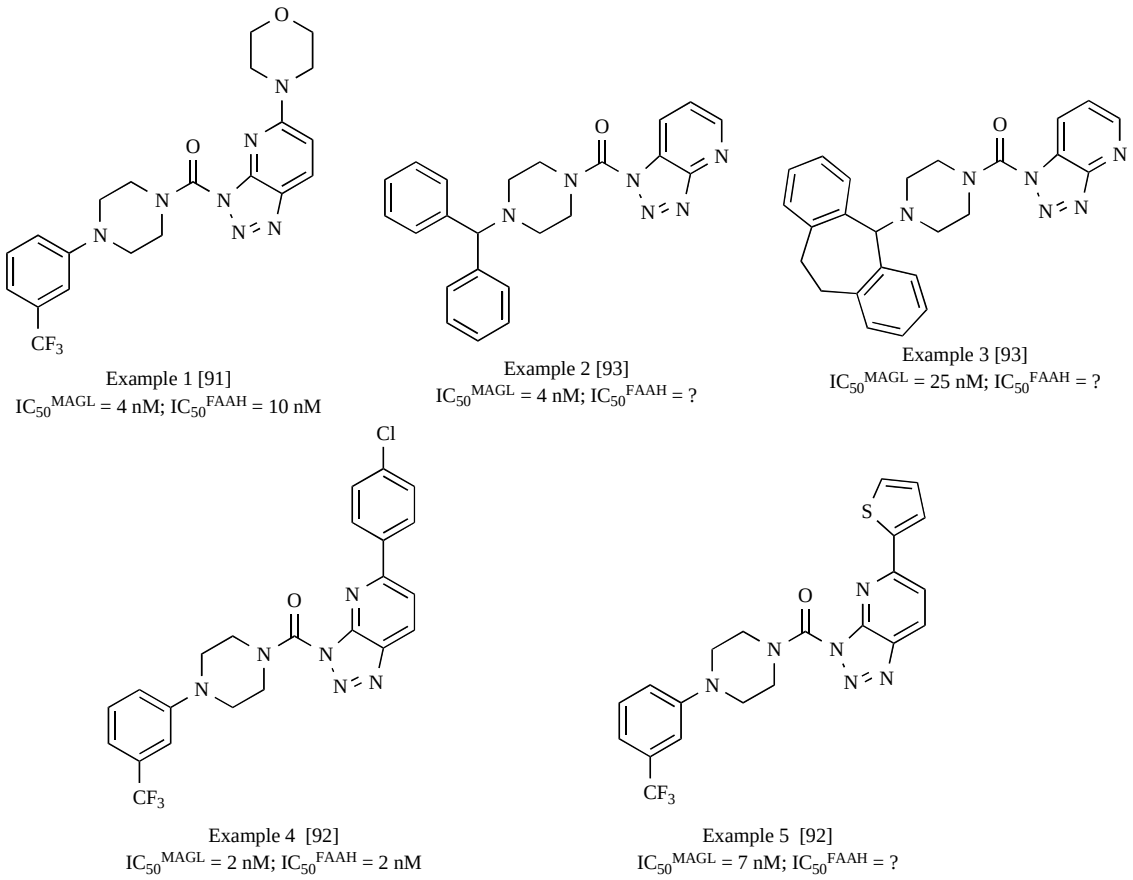

Fig. (8). Examples of triazolopyridine carboxamides and triazolopyrimidine carboxamides derivatives developed by Sanofi-Aventis group.

tor, additional undesirable targets (mainly lipases) might be identified in the future.

Apart from these issues, OMDM169 was tested *in vivo* for its antinociceptive properties. In the formalin test, the compound (2.5-5 mg/kg, i.p.) promoted analgesia in the second phase of nociception. This effect was accompanied by an increase in 2-AG levels in the ipsilateral paw that received formalin injection. However, 2-AG concentration without formalin injection did not change, likely reflecting the ability of the endocannabinoid biosynthesis pathways to respond "on-demand" to a stimulus, while remaining quiescent at basal state. Consistently, OMDM169 failed to elevate 2-AG level in untreated N18TG2 neuroblastoma in culture but increased its concentration when endocannabinoid biosynthesis was induced by ionomycin. This observation therefore supports the idea that inhibitors of endocannabinoid degradation could help to gain time or spatial selectivity over direct cannabinoid receptors agonists and avoid the central side effects associated with the use of the latter compounds. Unexpectedly enough given its *in vivo* phamacological effects, OMDM169 failed to produce a significant inhibition of 2-AG hydrolase activity in brain and paw skin from treated mice, reasons for this remaining unclear.

F. Following the success associated with the use of carbamates in the development of FAAH inhibitors [98], Hohmann and colleagues screened a library of *O*-biphenyl and *N*-biphenyl carbamates and reported the development of URB602 as the first selective inhibitor of 2-AG degradation, although its potency remained limited (IC_{50} = 28 μM and 223 μM on rat brain and pure recombinant rat MAGL, respectively) [70,99] (Fig. (**7**)). Despite the fact that it was shown to lead to the selective accumulation of 2-AG (not AEA) in rat hippocampal slices or after injection in the periaqueductal gray, this compound was subsequently reported to inhibit FAAH and MAGL with similar potencies [60,70,71,99]**.** Nevertheless, URB602 was used to demonstrate the implication of the endocannabinoid system in stress-induced analgesia and it exhibited CB_2-dependent analgesic and anti-inflammatory properties in a model of inflammation [100].

Other carbamates that lead to MAGL inhibition include SPB 01403 (IC_{50} = 31 μM on rat cerebellar membranes) [101], CAY10499 (IC_{50} = 0.5 μM to 20 nM on the pure human MAGL, depending on the preincubation time) [102], and 5-methoxy-3-(4-phenoxyphenyl)-3H-[1,3,4]oxadiazol-2-one (IC_{50} = 78 nM) [103] (Fig. (**7**)). However, SPB 01403 also inactivates FAAH with a higher affinity (IC_{50} = 0.52 μM on rat brain homogenates), and the two latter compounds lack selectivity over FAAH and, at least for CAY10499, the hormone sensitive lipase.

G. Without any doubt, one of the most promising breakthroughs in the quest for selective MAGL inhibitors came from the screening of a carbamate library and the development of a series of derivatives of this scaffold by Cravatt's team [61,104]. In an effort to optimize the selectivity during the development process, chemistry works were performed together with a biological evaluation using ABPP technology. This resulted in the low nanomolar inhibitor JZL184, which exhibits a comfortable selectivity versus FAAH *in vitro* (IC_{50} = 6 nM and 4 μM for recombinant MAGL and FAAH expressed in COS7 cells, respectively) (Fig. (**7**)). As it is the case for the 2,5-regioisomer of LY2183240, the inhibition mechanism involves the carbamoylation of the Ser122, with *p*-nitrophenol as the leaving group [62]. ABPP and enzymatic assays performed on brain membrane proteome from JZL184-treated mice confirmed the nearly complete and rather selective inhibition of MAGL and 2-AG hydrolysis (75% inhibition at 4 mg/kg). Encouragingly, ABPP-MudPIT also failed to identify other off-targets of JZL184 among the panel of 40 brain hydrolases that were tested. Note that, depending on the dose, FAAH was inhibited as well *in vivo*, although incompletely and to a lesser extent than MAGL. Noteworthy, ABPP-MudPIT experiment performed on lung membrane proteome allowed the identification of esterase 1, esterase 1-like and triacylglycerol hydrolase 2 as JZL184 off-targets, thus highlighting a still improvable selectivity [62]. Like OMDM169, the compound displays an interesting interspecies selectivity, being ten times more potent on human and mouse MAGL than on the rat ortholog. This is in contrast with OMDM169, which is more active on the human and rat enzyme, compared to the mouse ortholog.

As expected, JZL184 administration resulted in a dramatic concentration-dependent increase of brain 2-AG levels. Accumulation of *N*-acylethanolamines (in particular AEA) did not occur, unless at the highest dose (40 mg/kg). Using microdialysis, the authors also measured the signaling-competent concentrations of endocannabinoids and found that JZL184 enhances 2-AG pools in the synapsis, while leaving AEA levels unchanged. The accumulation of 2-AG *in vivo* is accompanied by a strong CB_1-dependent analgesic effect in different models of nociception (tail immersion, phase I and II of the formalin test, as well as acetic acid-induced tail writhing). Another key finding of this study is the observation that JZL184 administration results in a subset of effects belonging to the classical "tetrad", *i.e.* a CB_1 dependent hypothermia and hypomotility (but not catalepsy), thus sharing common properties with direct cannabinoid agonists, and above all rising crucial question regarding the clinical use of MAGL inhibitors.

3.10. Tridimensional Structure of MAGL

Secondary structure prediction softwares have allowed ranking MAGL among the α/β hydrolase fold family [45,49]. The α/β hydrolase fold is organized as a core domain constituted of a β-sheet made from eight β-strands (one of which is antiparallel to the others) and surrounded by a series of α helices. This central domain contains the catalytic triad, whose structural organization is well conserved. The nucleophilic serine is located at the top of the core in a typical sharp turn between α3 helix and β5 strand. This core domain is overhung by a much more variable domain termed cap, made of several α helices and loops that mediate structural information to provide the huge amount of α/β hydrolases family members with their specificity in both function and substrate profile.

In view of the absence of a crystallographic structure, several teams have reported structures of MAGL built by homology modelling [45,85,101,105]. These *in silico* models have revealed that three cysteines (Cys201, Cys208, Cys242, short isoform numbering), which are conserved between the human, rat and mouse orthologs, are located in the vicinity of the active site, close to the Ser122-His269-Asp239 triad [59]. While their functional role is unknown, it is known from mass spectroscopy experiments that these residues are not involved in disulfide bridge formation [106]. On the other hand, in the first study mentioning the characterization of purified MAGL, Tornqvist and Belfrage reported a more rapid loss of activity when the protein was incubated in absence of a reducing agent. Thus, there is a possibility that one or more of the cysteines play(s) a structural role in the stabilization of MAGL active conformation.

Besides this, Cys201, Cys208 and Cys242 also provided a rational basis for the development of maleimides as MAGL inhibitors [59], and it is likely that these residues could offer the opportunity to gain some selectivity for MAGL versus other serine hydrolases in the future. Indeed, recent mutagenesis studies have demonstrated that each of these cysteines can account for the inhibitory potency of several inhibitors. NAM inhibits irreversibly the enzyme through a Michael addition on either the Cys201 or Cys242 [80,85,107], whilst octhilinone acts by forming a partially reversible disulfide with Cys208 [85]. In addition, primisterin and euphol have been shown to interact reversibly with Cys208 and Cys201, respectively [86]. Docking studies have been performed using a MAGL tridimensional model to highlight the binding mode of these inhibitors [86].

Unfortunately, the closest homolog of known tridimensional structure, the chloroperoxidase L. from *S.lividans*, shares at best 20-25% sequence identity with MAGL. No significant similarity is found within the cap domain, which therefore constitutes a sizeable drawback of the homology modelling approach if one's aim is the design of MAGL inhibitors on a rational basis (Fig. (**9**)). Indeed, an *in silico* screening using a MAGL model has failed to produce valuable inhibitors of MAGL [101].

Recently, our group elucidated the tridimensional structure of the human MAGL by X ray diffaction, at the resolution of 2.2 Å (pdb entry, 3hju) [107]. The enzyme crystallizes as a dimer, and the structure confirms the enzyme as a member of the α/β hydrolase superfamily. It also directly evidenced the identity of the catalytic triad which had been proposed previously [45]. Simultaneously, Sanofi-Aventis group reported the crystal structure of the MAGL in complex with the inhibitor SAR629 [108].

```
MAGL                   1   mpeessprrt pqsipyqdlp hLVNADGQYL FCRYWKPtgt pka-------
Chloroperoxidase L.    1   mgt------- ---------- -VTTSDGTNI FYKDWGPrdg lpvvfhhgwp
                               β1                          β2          β3
MAGL                  44   ---------- LIFVSHGage hsgryeelar mlmgldllVF AHDHVGHGQS
Chloroperoxidase L.   33   lsaddwdnqm LFFLSHGyr- ---------- --------VI AHDRRGHGRS
                                     α1                    β4
MAGL                  84   Egermvvsdf hvfvrdvlqh vdsmqkdypg lpvflLGHSm -GGAIAILTA
Chloroperoxidase L.   64   Dqpstghdmd tyaadvaalt ealdlrgavh -----IGHSt gGGEVARYVA
                                           α2                β5          α3
MAGL                 133   AERPGHFAGM VLISPLVLAN PESATTfkvl aakvlnlvlp nlslgpidss
Chloroperoxidase L.  109   RAEPGRVAKA VLVSAVPPVM VKSDTNpdgl plevfdefra alaanraqfy
                                     β6                      α4
MAGL                 183   vlsrnktevd iynsdplicr aglkvcfgiq llnavsrver a---------
Chloroperoxidase L.  159   idvpsgpfyg fnregatvsq glidhwwlqg mmgaanahye ciaafsetdf
                           α5                α6                α7
MAGL                 224   ---LPKLTVP FLLLQGSADR Lcdskgayll melaksqDKT LKIYEGAYHV
Chloroperoxidase L.  209   tddLKRIDVP VLVAHGTDDQ Vvpyadaapk saella-NAT LKSYEGLPHG
                              α8        β7                          β8
MAGL                 271   LHKELPEVTN svfheinmwv sqrtatagta spp
Chloroperoxidase L.  258   MLSTHPEVLN pdllafvks- ---------- ---
                              α9        α10
```

Fig. (9). Alignment of the primary sequence of human MAGL with that of the chloroperoxidase from *Streptomyces lividans*, the closest homolog of known tridimensional structure. Only upper-case letters are considered to be aligned. The part of the sequence corresponding to the cap region is highlighted in a box. The catalytic triad is indicated in bold.

MAGL structure reveals several key features of the enzyme which are essential to explain its biological activity [107]. First, the structure differs substantially from that of the chloroperoxidase L (and several other haloperoxidases, esterases and other proteins from the superfamily) when considering the cap domain, as expected from the poor sequence homology in this region [109] (Fig. (**10**) and (Table **3**).

This part of the protein, which constitutes the active site entry, is organized as a wide U-shaped structure, which is to be compared to the narrower V-shaped device that is found in haloperoxidases and esterases. One of the edges of this entry is made of the hydrophobic α4-helix. These structural adaptations likely allow the MAGL to interact with the membranes and recruit the bulky and lipophilic substrate.

Within the active site, beside the catalytic triad, the oxyanion hole stabilizing the tetrahedral intermediate state during the hydrolysis is formed by Ala51 and Met123 backbone NHs.

The elucidation of the tridimensional structure of the apoenzyme and of a complex with an inhibitor also allows

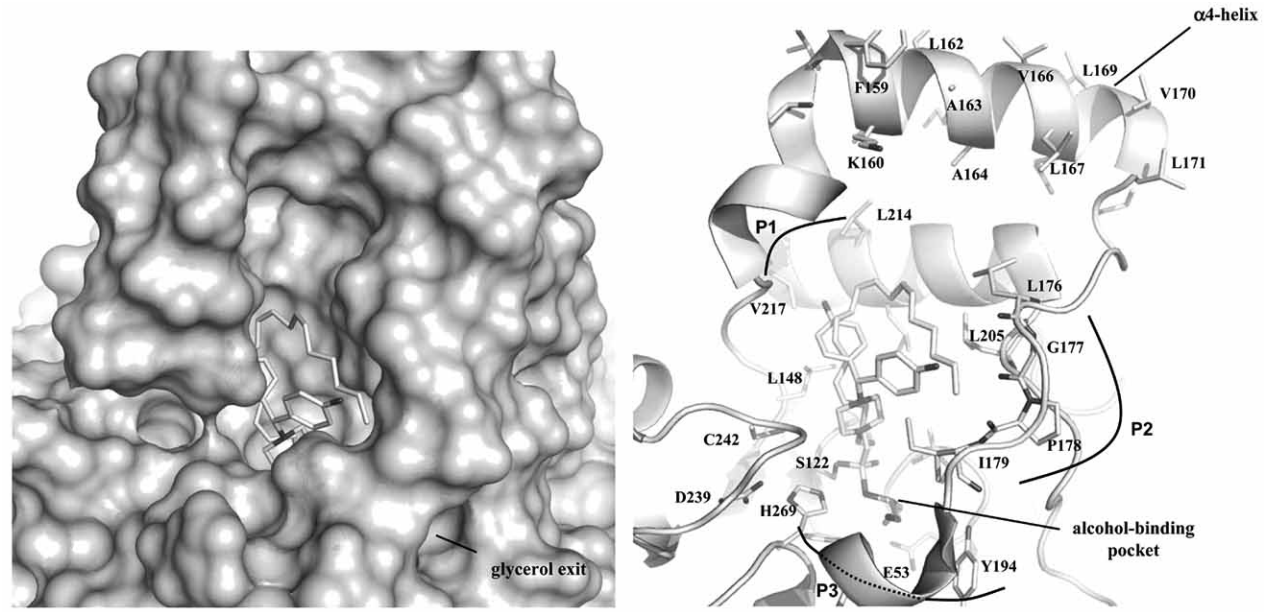

Fig. (10). Crystal structure of the human MAGL with 2-AG docked in the active site (pdb entry 3HJU) or in complex with SAR629 (pdb entry 3JWE). Left: surface representation. Right: the Ser122/His269/Asp239 catalytic triad and the main residues lining the sites that allow interaction with both ligands are represented as sticks. Two pockets filled with SAR629 fluorophenyl groups or with 2-AG acyl moiety (P1 and P2), as well as the alcohol binding pocket (P3), are highlighted. The hydrophobic α4-helix residues are also represented.

Table 3. Summary of the Main Structural Homologs of MAGL. Proteins with the Same Overall Fold in (Cap Region) are Pooled in the Same Box

Name	Organism	Pdb entry	References
Chloroperoxidase L.	*Streptomyces lividans*	1a88	[109]
Bromoperoxidase A1	*Streptomyces aureofaciens*	1a8q	[109]
Aryl esterase	*Pseudomonas fluorescens*	1va4	[110]
Stereoselective Esterase	*Pseudomonas putida*	1zoi	[111]
Bromoperoxidase	*Bacillus anthracis*	3fob	unpublished
Gamma-Lactamase	*Microbacterium*	1hkh	[112]
Sigma factor sigB regulation protein rsbQ	*Bacillus subtilis*	1wpr	[113]
…	…	…	…
Hypothetical protein PA2218	*Pseudomonas aeruginosa*	2hdw	unpublished
Carboxylesterase	*Bacillus stearothermophilus*	1r1d	unpublished
"Lipase-esterase related protein"	*Clostridium acetobutylicum* ATCC 824	3e0x	unpublished
human gastric lipase	*Homo sapiens*	1k8q	[114]

the identification of several sites that are important for the interaction with 2-AG and other ligands, and which could now reveal helpful for the design of new compounds (Fig. (**10**)). First, a wide acyl-binding tunnel bordered by numerous hydrophobic residues (*i.e.* Leu148, Leu176, Ile179, Leu205, Val207, Leu213, Leu214, Val217 and Leu241) delineates various pockets, which are filled by the fluorophenyl groups of SAR629 or by the acyl chain of 2-AG. More deeply buried in the active site, another pocket accommodates the 2-AG glycerol moiety. This alcohol-binding pocket has a more hydrophilic character and is delimited, among others, by the Tyr58 hydroxyl group, the NH from His121 and His272 side chains, the Arg57 guanidinium, the carboxylate from Glu53, and the carbonyl from Ala51.

Another important aspect for the knowledge of the MAGL concerns the role of the cap. In many lipases, it has evolved to function as a lid that alternates between a closed and an open conformation to allow the access to the active site [115]. Events that trigger opening of this lid include the interaction of the protein with lipid droplets, a process which is known as interfacial activation and may involve a cofactor, as in the case of the pancreatic lipase/colipase complex. Structures of lipases in an open confirmation were also favoured by the binding of lipids, the use of detergents as well as by the presence of inhibitors in the active site [114,116,117]. The human pancreatic lipase-related protein 2 has also been reported to crystallize in this open conformation without the need of a detergent or inhibitor [118]. Considering the MAGL, on the one hand, there is no biochemical evidence indicating that the interaction with the substrate would require such a structural reorganization of the cap. Besides this, the structure of the enzyme in complex with SAR629 does not present any major conformational difference compared to that of the apoenzyme. On the other hand, crystallographic evidence suggests that the cap is flexible and that alternative conformations might be possible [107,108]. Hence, further crystallographic studies will be needed to gain additional information about the functioning of this device.

4. CONCLUSION

Although the validation of 2-AG signaling pathway as a pharmaceutical target for drug development has long suffered the paucity of the available inhibitors, evidence has converged to support the crucial role of this endocannabinoid in retrograde synaptic transmission. The determinant implication of MAGL in this process as well as preliminary pharmacological tests conducted both *in vitro* and *in vivo* underline the interest of MAGL inhibition to treat several pathological disorders such as pain and cancer. However, numerous challenges remain.

First, whilst the recent development of JZL184 allowed the cannabinoid community to be in a good position to answer the crucial question of the physiological meaning of MAGL, definite evidence still waits the knowledge of the consequences of MAGL gene invalidation. Second, the understanding of putative regulatory processes for MAGL activity is still in the infancy. In particular, what is the significance of the distinct MAGL forms observed in several tissues?

Is MAGL a drug target? The observation that mice injected with JZL184 elicit several of the effects classically associated with CB_1 cannabinoid receptors direct agonists is of major concern. Beside the fact that it is established that endocannabinoid biosynthesis responds "on demand" to a stimulus or stress and that FAAH inhibition does not result in cannabinoid-like effects, this worrying finding casts some doubt on the idea that targeting MAGL would allow to circumvent the problems associated with the use of direct cannabinoid agonists, by enhancing the endocannaboid activity "where and when needed".

To answer these fundamental questions, new tools are required. Apart from $MAGL^{-/-}$ mice model, the development of JZL184 and a few other compounds has constituted a first step towards the design of more potent and more selective inhibitors.

Along this line, the recent availability of the MAGL tridimensional structure renders conceivable the rational de-

sign of inhibitors. *De novo* design, *in silico* screening, and optimization of existing compounds (using docking or crystal structure of enzyme-inhibitor complexes) are imaginable using computational and experimental approaches. In particular, it could help the development of selective compounds that would target the MAGL without interacting with FAAH. Since both proteins are structurally distant from each other, making advantage of several features of the MAGL – *i.e.* the cysteines and their environment, the wide hydrophobic channel and the hydrophilic pocket – should help to solve the selectivity issues that have been encountered with most of the known MAGL inhibitors.

Besides this, the structure gives an insight into the cap organization, the substrate recognition process, and potential dynamic events regulating MAGL activity. Now, it also opens new questions, and a lot of work has still to be carried out in this field. Indeed, the determination of the structure of the apoenzyme only constitutes a first step towards a more in depth knowledge of the enzyme. What is the function of the cap? Does is behave as a lid or is it constitutively open?

Similarly, what is the role of three cysteines in the active site and cap domain? Do they play a structural role or are they regulatory devices to modulate the enzyme activity? Which structural characteristics account for the interspecies differences observed for the inhibitory potency of OMDM169 and JZL184 or for the MAGL preference for unsaturated substrates, for instance?

Prospects -and challenges- are legion. Hopefully, will the tridimensional structure and newly developed inhibitors constitute useful tools for the future.

ABBREVIATIONS

AEA	=	anandamide
2-AG	=	2-arachidonoylglycerol
ATGL	=	adipose tissue triacylglycerol hydrolase
DAG	=	diacylglycerol
DSE	=	depolarization-induced suppression of excitation
DSI	=	depolarization-induced suppression of inhibition
EPSC	=	excitatory post-synaptic currents
FAAH	=	fatty acid amide hydrolase
HSL	=	hormone sensitive lipase
IPSC	=	inhibitory post-synaptic currents
MAGL	=	monoacylglycerol lipase
PKA	=	protein kinase A
PPAR	=	peroxisome proliferator-activated receptor
Δ^9-THC	=	Δ^9-tetrahydrocannabinol
MAG	=	monoacylglycerol
DAG	=	diacylglycerol
TAG	=	triacylglycerol

REFERENCES

[1] Russo, E. B. History of cannabis and its preparations in saga, science, and sobriquet. *Chem. Biodivers.*, **2007**, *4*, 1614-48.

[2] Gaoni, Y.; Mechoulam, R. Isolation, structure and partial synthesis of an active constituant of hashish. *J. Am. Chem. Soc.*, **1964**, *86*, 1646-7.

[3] Mechoulam, R.; Feigenbaum, J. J. Towards cannabinoid drugs. *Prog. Med. Chem.*, **1987**, *24*, 159-207.

[4] Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.*, **1988**, *247*, 1046-51.

[5] Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, **1990**, *346*, 561-4.

[6] Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **1993**, *365*, 61-5.

[7] Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, **1992**, *258*, 1946-9.

[8] Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.*, **1995**, *215*, 89-97.

[9] Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.*, **1995**, *50*, 83-90.

[10] Di Marzo, V. The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol. Res.*, **2009**, *60*, 77-84.

[11] Di Marzo, V; Petrosino, S. Endocannabinoids and the regulation of their levels in health and disease. *Curr. Opin. Lipidol.*, **2007**, *18*, 129-40.

[12] Howlett, A. C. Cannabinoid receptor signaling. *Handb. Exp. Pharmacol.*, **2005**, 53-79.

[13] Kano, M.; Ohno-Shosaku, T.; Hashimotodani, Y.; Uchigashima, M.; Watanabe, M. Endocannabinoid-mediated control of synaptic transmission. *Physiol. Rev.*, **2009**, *89*, 309-80.

[14] Pacher, P.; Batkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.*, **2006**, *58*, 389-462.

[15] Liu, J.; Wang, L.; Harvey-White, J.; Huang, B. X.; Kim, H. Y.; Luquet, S.; Palmiter, R. D.; Krystal, G.; Rai, R.; Mahadevan, A.; Razdan, R. K.; Kunos, G. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology*, **2008**, *54*, 1-7.

[16] Sugiura, T.; Kishimoto, S.; Oka, S.; Gokoh, M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog. Lipid Res.*, **2006**, *45*, 405-46.

[17] Wang, J.; Ueda, N. Biology of endocannabinoid synthesis system. *Prostaglandins Other Lipid Mediat.*, **2009**, *89*, 112-9.

[18] De Petrocellis, L.; Di Marzo, V. An introduction to the endocannabinoid system: from the early to the latest concepts. *Best. Pract. Res. Clin. Endocrinol. Metab*, **2009**, *23*, 1-15.

[19] Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature*, **1996**, *384*, 83-7.

[20] Cravatt, B. F.; Demarest, K.; Patricelli, M. P.; Bracey, M. H.; Giang, D. K.; Martin, B. R.; Lichtman, A. H. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc. Natl. Acad. Sci. U.S.A.*, **2001**, *98*, 9371-6.

[21] Lafontan, M.; Langin, D. Lipolysis and lipid mobilization in human adipose tissue. *Prog. Lipid Res.*, **2009**, *48*, 275-97.

[22] Vaughan, M.; Berger, J. E.; Steinberg, D. Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue. *J. Biol. Chem.*, **1964**, *239*, 401-9.

[23] Fredrikson, G.; Stralfors, P.; Nilsson, N. O.; Belfrage, P. Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J. Biol. Chem.*, **1981**, *256*, 6311-20.

[24] Zechner, R.; Kienesberger, P. C.; Haemmerle, G.; Zimmermann, R.; Lass, A. Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *J. Lipid Res.*, **2009**, *50*, 3-21.

[25] Fredrikson, G.; Belfrage, P. Positional specificity of hormone-sensitive lipase from rat adipose tissue. *J. Biol. Chem.*, **1983**, *258*, 14253-6.

[26] Fredrikson, G.; Tornqvist, H.; Belfrage, P. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim. Biophys. Acta*, **1986**, *876*, 288-93.

[27] Berglund, L.; Khoo, J. C.; Jensen, D.; Steinberg, D. Resolution of hormone-sensitive triglyceride/diglyceride lipase from monoglyceride lipase of chicken adipose tissue. *J. Biol. Chem.*, **1980**, *255*, 5420-8.

[28] Stralfors, P.; Belfrage, P. Phosphorylation of hormone-sensitive lipase by cyclic AMP-dependent protein kinase. *J. Biol. Chem.*, **1983**, *258*, 15146-52.

[29] Belfrage, P.; Fredrikson, G.; Nilsson, N. O.; Stralfors, P. Regulation of adipose tissue lipolysis: phosphorylation of hormones sensitive lipase in intact rat adipocytes. *FEBS Lett.*, **1980**, *111*, 120-4.

[30] Nilsson, N. O.; Stralfors, P.; Fredrikson, G.; Belfrage, P. Regulation of adipose tissue lipolysis: effects of noradrenaline and insulin on phosphorylation of hormone-sensitive lipase and on lipolysis in intact rat adipocytes. *FEBS Lett.*, **1980**, *111*, 125-30.

[31] Osuga, J.; Ishibashi, S.; Oka, T.; Yagyu, H.; Tozawa, R.; Fujimoto, A.; Shionoiri, F.; Yahagi, N.; Kraemer, F. B.; Tsutsumi, O.; Yamada, N. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. *Proc. Natl. Acad. Sci. U.S.A.*, **2000**, *97*, 787-92.

[32] Haemmerle, G.; Zimmermann, R.; Hayn, M.; Theussl, C.; Waeg, G.; Wagner, E.; Sattler, W.; Magin, T. M.; Wagner, E. F.; Zechner, R. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. *J. Biol. Chem.*, **2002**, *277*, 4806-15.

[33] Wang, S. P.; Laurin, N.; Himms-Hagen, J.; Rudnicki, M. A.; Levy, E.; Robert, M. F.; Pan, L.; Oligny, L.; Mitchell, G. A. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes. Res.*, **2001**, *9*, 119-28.

[34] Zimmermann, R.; Strauss, J. G.; Haemmerle, G.; Schoiswohl, G.; Birner-Gruenberger, R.; Riederer, M.; Lass, A.; Neuberger, G.; Eisenhaber, F.; Hermetter, A.; Zechner, R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*, **2004**, *306*, 1383-6.

[35] Haemmerle, G.; Lass, A.; Zimmermann, R.; Gorkiewicz, G.; Meyer, C.; Rozman, J.; Heldmaier, G.; Maier, R.; Theussl, C.; Eder, S.; Kratky, D.; Wagner, E. F.; Klingenspor, M.; Hoefler, G.; Zechner, R. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. *Science*, **2006**, *312*, 734-7.

[36] Festuccia, W. T.; Laplante, M.; Berthiaume, M.; Gelinas, Y.; Deshaies, Y. PPARgamma agonism increases rat adipose tissue lipolysis, expression of glyceride lipases, and the response of lipolysis to hormonal control. *Diabetologia*, **2006**, *49*, 2427-36.

[37] Kershaw, E. E.; Schupp, M.; Guan, H. P.; Gardner, N. P.; Lazar, M. A.; Flier, J. S. PPARgamma regulates adipose triglyceride lipase in adipocytes *in vitro* and *in vivo*. *Am. J. Physiol. Endocrinol. Metab.*, **2007**, *293*, E1736-E1745.

[38] Subramanian, V.; Rothenberg, A.; Gomez, C.; Cohen, A. W.; Garcia, A.; Bhattacharyya, S.; Shapiro, L.; Dolios, G.; Wang, R.; Lisanti, M. P.; Brasaemle, D. L. Perilipin A mediates the reversible binding of CGI-58 to lipid droplets in 3T3-L1 adipocytes. *J. Biol. Chem.*, **2004**, *279*, 42062-71.

[39] Yamaguchi, T.; Omatsu, N.; Matsushita, S.; Osumi, T. CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome. *J. Biol. Chem.*, **2004**, *279*, 30490-7.

[40] Granneman, J. G.; Moore, H. P.; Granneman, R. L.; Greenberg, A. S.; Obin, M. S.; Zhu, Z. Analysis of lipolytic protein trafficking and interactions in adipocytes. *J. Biol. Chem.*, **2007**, *282*, 5726-35.

[41] Granneman, J. G.; Moore, H. P.; Krishnamoorthy, R.; Rathod, M. Perilipin controls lipolysis by regulating the interactions of ab-hydrolase containing 5 (Abhd5) and adipose triglyceride lipase (ATGL). *J. Biol. Chem.*, **2009**.

[42] Zimmermann, R.; Lass, A.; Haemmerle, G.; Zechner, R. Fate of fat: the role of adipose triglyceride lipase in lipolysis. *Biochim. Biophys. Acta*, **2009**, *1791*, 494-500.

[43] Kupiecki, F. P. Partial purification of monoglyceride lipase from adipose tissue. *J. Lipid Res.*, **1966**, *7*, 230-5.

[44] Tornqvist, H.; Belfrage, P. Purification and some properties of a monoacylglycerol-hydrolyzing enzyme of rat adipose tissue. *J. Biol. Chem.*, **1976**, *251*, 813-9.

[45] Karlsson, M.; Contreras, J. A.; Hellman, U.; Tornqvist, H.; Holm, C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J. Biol. Chem.*, **1997**, *272*, 27218-23.

[46] Karlsson, M.; Reue, K.; Xia, Y. R.; Lusis, A. J.; Langin, D.; Tornqvist, H.; Holm, C. Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene. *Gene*, **2001**, *272*, 11-8.

[47] Wall, E. M.; Cao, J.; Chen, N.; Buller, R. M.; Upton, C. A novel poxvirus gene and its human homolog are similar to an E. coli lysophospholipase. *Virus Res.*, **1997**, *52*, 157-67.

[48] Arpigny, J. L.; Jaeger, K. E. Bacterial lipolytic enzymes: classification and properties. *Biochem. J.*, **1999**, *343 Pt 1*, 177-83.

[49] Ollis, D. L.; Cheah, E.; Cygler, M.; Dijkstra, B.; Frolow, F.; Franken, S. M.; Harel, M.; Remington, S. J.; Silman, I.; Schrag, J.; . The alpha/beta hydrolase fold. *Protein Eng.*, **1992**, *5*, 197-211.

[50] Karlsson, M.; Tornqvist, H.; Holm, C. Expression, purification, and characterization of histidine-tagged mouse monoglyceride lipase from baculovirus-infected insect cells. *Protein Expr. Purif.*, **2000**, *18*, 286-92.

[51] Vila, A.; Rosengarth, A.; Piomelli, D.; Cravatt, B.; Marnett, L. J. Hydrolysis of prostaglandin glycerol esters by the endocannabinoid-hydrolyzing enzymes, monoacylglycerol lipase and fatty acid amide hydrolase. *Biochemistry*, **2007**, *46*, 9578-85.

[52] Rindlisbacher, B.; Reist, M.; Zahler, P. Diacylglycerol breakdown in plasma membranes of bovine chromaffin cells is a two-step mechanism mediated by a diacylglycerol lipase and a monoacylglycerol lipase. *Biochim. Biophys. Acta*, **1987**, *905*, 349-57.

[53] Vandevoorde, S.; Saha, B.; Mahadevan, A.; Razdan, R. K.; Pertwee, R. G.; Martin, B. R.; Fowler, C. J. Influence of the degree of unsaturation of the acyl side chain upon the interaction of analogues of 1-arachidonoylglycerol with monoacylglycerol lipase and fatty acid amide hydrolase. *Biochem. Biophys. Res. Commun.*, **2005**, *337*, 104-9.

[54] Ghafouri, N.; Tiger, G.; Razdan, R. K.; Mahadevan, A.; Pertwee, R. G.; Martin, B. R.; Fowler, C. J. Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. *Br. J. Pharmacol.*, **2004**, *143*, 774-84.

[55] Dinh, T. P.; Carpenter, D.; Leslie, F. M.; Freund, T. F.; Katona, I.; Sensi, S. L.; Kathuria, S.; Piomelli, D. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci. U. S. A*, **2002**, *99*, 10819-24.

[56] Gulyas, A. I.; Cravatt, B. F.; Bracey, M. H.; Dinh, T. P.; Piomelli, D.; Boscia, F.; Freund, T. F. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.*, **2004**, *20*, 441-58.

[57] Blankman, J. L.; Simon, G. M.; Cravatt, B. F. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.*, **2007**, *14*, 1347-56.

[58] Dinh, T. P.; Kathuria, S.; Piomelli, D. RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. *Mol. Pharmacol.*, **2004**, *66*, 1260-4.

[59] Saario, S. M.; Salo, O. M.; Nevalainen, T.; Poso, A.; Laitinen, J. T.; Jarvinen, T.; Niemi, R. Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. *Chem. Biol.*, **2005**, *12*, 649-56.

[60] Vandevoorde, S.; Jonsson, K. O.; Labar, G.; Persson, E.; Lambert, D. M.; Fowler, C. J. Lack of selectivity of URB602 for

2-oleoylglycerol compared to anandamide hydrolysis *in vitro*. *Br. J. Pharmacol.*, **2007**, *150*, 186-91.

[61] Long, J. Z.; Li, W.; Booker, L.; Burston, J. J.; Kinsey, S. G.; Schlosburg, J. E.; Pavon, F. J.; Serrano, A. M.; Selley, D. E.; Parsons, L. H.; Lichtman, A. H.; Cravatt, B. F. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat. Chem. Biol.*, **2009**, *5*, 37-44.

[62] Long, J. Z.; Nomura, D. K.; Cravatt, B. F. Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. *Chem. Biol.*, **2009**, *16*, 744-53.

[63] Rakhshandehroo, M.; Sanderson, L. M.; Matilainen, M.; Stienstra, R.; Carlberg, C.; de Groot, P. J.; Muller, M.; Kersten, S. Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid metabolism by expression profiling. *PPAR. Res.*, **2007**, *2007*, 26839.

[64] Di Marzo, V. CB(1) receptor antagonism: biological basis for metabolic effects. *Drug Discov. Today*, **2008**, *13*, 1026-41.

[65] Patel, S.; Carrier, E. J.; Ho, W. S.; Rademacher, D. J.; Cunningham, S.; Reddy, D. S.; Falck, J. R.; Cravatt, B. F.; Hillard, C. J. The postmortal accumulation of brain N-arachidonylethanolamine (anandamide) is dependent upon fatty acid amide hydrolase activity. *J. Lipid Res.*, **2005**, *46*, 342-9.

[66] Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La, R. G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.*, **2003**, *9*, 76-81.

[67] Fegley, D.; Gaetani, S.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; Piomelli, D. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. *J. Pharmacol. Exp. Ther.*, **2005**, *313*, 352-8.

[68] Stella, N.; Piomelli, D. Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *Eur. J. Pharmacol.*, **2001**, *425*, 189-96.

[69] Wilson, R. I.; Nicoll, R. A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*, **2001**, *410*, 588-92.

[70] Hohmann, A. G.; Suplita, R. L.; Bolton, N. M.; Neely, M. H.; Fegley, D.; Mangieri, R.; Krey, J. F.; Walker, J. M.; Holmes, P. V.; Crystal, J. D.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; Piomelli, D. An endocannabinoid mechanism for stress-induced analgesia. *Nature*, **2005**, *435*, 1108-12.

[71] Makara, J. K.; Mor, M.; Fegley, D.; Szabo, S. I.; Kathuria, S.; Astarita, G.; Duranti, A.; Tontini, A.; Tarzia, G.; Rivara, S.; Freund, T. F.; Piomelli, D. Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. *Nat. Neurosci.*, **2005**, *8*, 1139-41.

[72] Kim, J.; Alger, B. E. Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nat. Neurosci.*, **2004**, *7*, 697-8.

[73] Hashimotodani, Y.; Ohno-Shosaku, T.; Kano, M. Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus. *J. Neurosci.*, **2007**, *27*, 1211-9.

[74] Pan, B.; Wang, W.; Long, J. Z.; Sun, D.; Hillard, C. J.; Cravatt, B. F.; Liu, Q. S. Blockade of 2-arachidonoylglycerol hydrolysis by selective monoacylglycerol lipase inhibitor 4-nitrophenyl 4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184) Enhances retrograde endocannabinoid signaling. *J. Pharmacol. Exp. Ther.*, **2009**, *331*, 591-7.

[75] Straiker, A.; Hu, S. S.; Long, J. Z.; Arnold, A.; Wager-Miller, J.; Cravatt, B. F.; Mackie, K. Monoacylglycerol lipase limits the duration of endocannabinoid-mediated depolarization-induced suppression of excitation in autaptic hippocampal neurons. *Mol. Pharmacol.*, **2009**, *76*, 1220-7.

[76] Chevaleyre, V.; Castillo, P. E. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron*, **2003**, *38*, 461-72.

[77] Safo, P. K.; Regehr, W. G. Endocannabinoids control the induction of cerebellar LTD. *Neuron*, **2005**, *48*, 647-59.

[78] Hashimotodani, Y.; Ohno-Shosaku, T.; Kano, M. Endocannabinoids and synaptic function in the CNS. *Neuroscientist*, **2007**, *13*, 127-37.

[79] Nomura, D. K.; Long, J. Z.; Niessen, S.; Hoover, H. S.; Ng, S. W.; Cravatt, B. F. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell*, **2010**, *140*, 49-61.

[80] Zvonok, N.; Pandarinathan, L.; Williams, J.; Johnston, M.; Karageorgos, I.; Janero, D. R.; Krishnan, S. C.; Makriyannis, A. Covalent inhibitors of human monoacylglycerol lipase: ligand-assisted characterization of the catalytic site by mass spectrometry and mutational analysis. *Chem. Biol.*, **2008**, *15*, 854-62.

[81] Matuszak, N.; Muccioli, G. G.; Labar, G.; Lambert, D. M. Synthesis and *in vitro* evaluation of N-substituted maleimide derivatives as selective monoglyceride lipase inhibitors. *J. Med. Chem.*, **2009**, *52*, 7410-20.

[82] Burston, J. J.; Sim-Selley, L. J.; Harloe, J. P.; Mahadevan, A.; Razdan, R. K.; Selley, D. E.; Wiley, J. L. N-arachidonyl maleimide potentiates the pharmacological and biochemical effects of the endocannabinoid 2-arachidonylglycerol through inhibition of monoacylglycerol lipase. *J. Pharmacol. Exp. Ther.*, **2008**, *327*, 546-53.

[83] Labar, G.; Bauvois, C.; Muccioli, G. G.; Wouters, J.; Lambert, D. M. Disulfiram is an inhibitor of human purified monoacylglycerol lipase, the enzyme regulating 2-arachidonoylglycerol signaling. *Chembiochem.*, **2007**, *8*, 1293-7.

[84] Kapanda, C. N.; Muccioli, G. G.; Labar, G.; Poupaert, J. H.; Lambert, D. M. Bis(dialkylaminethiocarbonyl)disulfides as potent and selective monoglyceride lipase inhibitors. *J. Med. Chem.*, **2009**, *52*, 7310-4.

[85] King, A. R.; Lodola, A.; Carmi, C.; Fu, J.; Mor, M.; Piomelli, D. A critical cysteine residue in monoacylglycerol lipase is targeted by a new class of isothiazolinone-based enzyme inhibitors. *Br. J. Pharmacol.*, **2009**, *157*, 974-83.

[86] King, A. R.; Dotsey, E. Y.; Lodola, A.; Jung, K. M.; Ghomian, A.; Qiu, Y.; Fu, J.; Mor, M.; Piomelli, D. Discovery of potent and reversible monoacylglycerol lipase inhibitors. *Chem. Biol.*, **2009**, *16*, 1045-52.

[87] Nithipatikom, K.; Endsley, M. P.; Isbell, M. A.; Falck, J. R.; Iwamoto, Y.; Hillard, C. J.; Campbell, W. B. 2-arachidonoylglycerol: a novel inhibitor of androgen-independent prostate cancer cell invasion. *Cancer Res.*, **2004**, *64*, 8826-30.

[88] Nithipatikom, K.; Endsley, M. P.; Isbell, M. A.; Wheelock, C. E.; Hammock, B. D.; Campbell, W. B. A new class of inhibitors of 2-arachidonoylglycerol hydrolysis and invasion of prostate cancer cells. *Biochem. Biophys. Res. Commun.*, **2005**, *332*, 1028-33.

[89] Boger, D. L.; Sato, H.; Lerner, A. E.; Austin, B. J.; Patterson, J. E.; Patricelli, M. P.; Cravatt, B. F. Trifluoromethyl ketone inhibitors of fatty acid amide hydrolase: a probe of structural and conformational features contributing to inhibition. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 265-70.

[90] Moore, S. A.; Nomikos, G. G.; Dickason-Chesterfield, A. K.; Schober, D. A.; Schaus, J. M.; Ying, B. P.; Xu, Y. C.; Phebus, L.; Simmons, R. M.; Li, D.; Iyengar, S.; Felder, C. C. Identification of a high-affinity binding site involved in the transport of endocannabinoids. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 17852-7.

[91] Alexander, J. P.; Cravatt, B. F. The putative endocannabinoid transport blocker LY2183240 is a potent inhibitor of FAAH and several other brain serine hydrolases. *J. Am. Chem. Soc.*, **2006**, *128*, 9699-704.

[92] Maione, S.; Morera, E.; Marabese, I.; Ligresti, A.; Luongo, L.; Ortar, G.; Di Marzo, V. Antinociceptive effects of tetrazole inhibitors of endocannabinoid inactivation: cannabinoid and non-cannabinoid receptor-mediated mechanisms. *Br. J. Pharmacol.*, **2008**, *155*, 775-82.

[93] Ortar, G.; Cascio, M. G.; Moriello, A. S.; Camalli, M.; Morera, E.; Nalli, M.; Di Marzo, V. Carbamoyl tetrazoles as inhibitors of endocannabinoid inactivation: a critical revisitation. *Eur. J. Med. Chem.*, **2008**, *43*, 62-72.

[94] Even; Hoornaert. Triazolopyridine carboxamide derivatives and triazolopyrimidine carboxamide derivatives, preparation thereof and therapeutic use thereof. WO 2008/145839, PCT/FR2008/000532., 2008.

[95] Even ; Hoornaert. Triazolopyridine carboxamide derivatives and triazolopyrimidine carboxamide derivatives, preparation thereof and therapeutic use thereof. WO 2008/145843, PCT/FR2008/000536, 2008.

[96] Hoornaert. Triazolopyridine carboxamide derivatives, preparation thereof and therapeutic use thereof. **W**O 2008/145842, PCT/FR2008/000535, 2008.

[97] Bisogno, T.; Ortar, G.; Petrosino, S.; Morera, E.; Palazzo, E.; Nalli, M.; Maione, S.; Di Marzo, V. Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity *in vivo*. *Biochim. Biophys. Acta*, **2009**, *1791*, 53-60.

[98] Labar, G.; Michaux, C. Fatty acid amide hydrolase: from characterization to therapeutics. *Chem. Biodivers.*, **2007**, *4*, 1882-902.

[99] King, A. R.; Duranti, A.; Tontini, A.; Rivara, S.; Rosengarth, A.; Clapper, J. R.; Astarita, G.; Geaga, J. A.; Luecke, H.; Mor, M.; Tarzia, G.; Piomelli, D. URB602 inhibits monoacylglycerol lipase and selectively blocks 2-arachidonoylglycerol degradation in intact brain slices. *Chem. Biol.*, **2007**, *14*, 1357-65.

[100] Comelli, F.; Giagnoni, G.; Bettoni, I.; Colleoni, M.; Costa, B. The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation. *Br. J. Pharmacol.*, **2007**, *152*, 787-94.

[101] Saario, S. M.; Poso, A.; Juvonen, R. O.; Jarvinen, T.; Salo-Ahen, O. M. Fatty acid amide hydrolase inhibitors from virtual screening of the endocannabinoid system. *J. Med. Chem.*, **2006**, *49*, 4650-6.

[102] Muccioli, G. G.; Labar, G.; Lambert, D. M. CAY10499, a novel monoglyceride lipase inhibitor evidenced by an expeditious MGL assay. *Chembiochem*, **2008**, *9*, 2704-10.

[103] Minkkila, A.; Savinainen, J. R.; Kasnanen, H.; Xhaard, H.; Nevalainen, T.; Laitinen, J. T.; Poso, A.; Leppanen, J.; Saario, S. M. Screening of various hormone-sensitive lipase inhibitors as endocannabinoid-hydrolyzing enzyme inhibitors. *ChemMedChem*, **2009**, *4*, 1253-9.

[104] Li, W.; Blankman, J. L.; Cravatt, B. F. A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases. *J. Am. Chem. Soc.*, **2007**, *129*, 9594-5.

[105] Bowman, A. L.; Makriyannis, A. Refined homology model of monoacylglycerol lipase: toward a selective inhibitor. *J. Comput. Aided Mol. Des*, **2009**, *23*, 799-806.

[106] Zvonok, N.; Williams, J.; Johnston, M.; Pandarinathan, L.; Janero, D. R.; Li, J.; Krishnan, S. C.; Makriyannis, A. Full mass spectrometric characterization of human monoacylglycerol lipase generated by large-scale expression and single-step purification. *J. Proteome. Res.*, **2008**, *7*, 2158-64.

[107] Labar, G.; Bauvois, C.; Borel, F.; Ferrer, J. L.; Wouters, J.; Lambert, D. M. Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. *Chembiochem*, **2010**, *11*, 218-27.

[108] Bertrand, T.; Auge, F.; Houtmann, J.; Rak, A.; Vallee, F.; Mikol, V.; Berne, P. F.; Michot, N.; Cheuret, D.; Hoornaert, C.; Mathieu, M. Structural basis for human monoglyceride lipase inhibition. *J. Mol. Biol.*, **2010**, *396*, 663-73.

[109] Hofmann, B.; Tolzer, S.; Pelletier, I.; Altenbuchner, J.; van Pee, K. H.; Hecht, H. J. Structural investigation of the cofactor-free chloroperoxidases. *J. Mol. Biol.*, **1998**, *279*, 889-900.

[110] Cheeseman, J. D.; Tocilj, A.; Park, S.; Schrag, J. D.; Kazlauskas, R. J. Structure of an aryl esterase from Pseudomonas fluorescens. *Acta Crystallogr. D. Biol. Crystallogr.*, **2004**, *60*, 1237-43.

[111] Elmi, F.; Lee, H. T.; Huang, J. Y.; Hsieh, Y. C.; Wang, Y. L.; Chen, Y. J.; Shaw, S. Y.; Chen, C. J. Stereoselective esterase from Pseudomonas putida IFO12996 reveals alpha/beta hydrolase folds for D-beta-acetylthioisobutyric acid synthesis. *J. Bacteriol.*, **2005**, *187*, 8470-6.

[112] Line, K.; Isupov, M. N.; Littlechild, J. A. The crystal structure of a (-) gamma-lactamase from an Aureobacterium species reveals a tetrahedral intermediate in the active site. *J. Mol. Biol.*, **2004**, *338*, 519-32.

[113] Kaneko, T.; Tanaka, N.; Kumasaka, T. Crystal structures of RsbQ, a stress-response regulator in Bacillus subtilis. *Protein Sci.*, **2005**, *14*, 558-65.

[114] Roussel, A.; Miled, N.; Berti-Dupuis, L.; Riviere, M.; Spinelli, S.; Berna, P.; Gruber, V.; Verger, R.; Cambillau, C. Crystal structure of the open form of dog gastric lipase in complex with a phosphonate inhibitor. *J. Biol. Chem.*, **2002**, *277*, 2266-74.

[115] Brzozowski, A. M.; Derewenda, U.; Derewenda, Z. S.; Dodson, G. G.; Lawson, D. M.; Turkenburg, J. P.; Bjorkling, F.; Huge-Jensen, B.; Patkar, S. A.; Thim, L. A model for interfacial activation in lipases from the structure of a fungal lipase-inhibitor complex. *Nature*, **1991**, *351*, 491-4.

[116] Egloff, M. P.; Marguet, F.; Buono, G.; Verger, R.; Cambillau, C.; van Tilbeurgh, H. The 2.46 A resolution structure of the pancreatic lipase-colipase complex inhibited by a C11 alkyl phosphonate. *Biochemistry*, **1995**, *34*, 2751-62.

[117] van Tilbeurgh, H.; Gargouri, Y.; Dezan, C.; Egloff, M. P.; Nesa, M. P.; Ruganie, N.; Sarda, L.; Verger, R.; Cambillau, C. Crystallization of pancreatic procolipase and of its complex with pancreatic lipase. *J. Mol. Biol.*, **1993**, *229*, 552-4.

[118] Eydoux, C.; Spinelli, S.; Davis, T. L.; Walker, J. R.; Seitova, A.; Dhe-Paganon, S.; De Caro, A.; Cambillau, C.; Carriere, F. Structure of human pancreatic lipase-related protein 2 with the lid in an open conformation. *Biochemistry*, **2008**, *47*, 9553-64.

Received: February 15, 2010 **Revised: May 16, 2010** **Accepted: May 18, 2010**