Progress in Lipid Research 71 (2018) 1–17

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

2-Arachidonoylglycerol: A signaling lipid with manifold actions in the brain

Marc P. Baggelaar[a,1], Mauro Maccarrone[b,c,2], Mario van der Stelt[a,*,2]

[a] Department of Molecular Physiology, Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands.
[b] Department of Medicine, Campus Bio-Medico University of Rome, Via Alvaro del Portillo 21, 00128 Rome, Italy
[c] European Centre for Brain Research/IRCCS Santa Lucia Foundation, via del Fosso del Fiorano 65, 00143 Rome, Italy

ABSTRACT

2-Arachidonoylglycerol (2-AG) is a signaling lipid in the central nervous system that is a key regulator of neurotransmitter release. 2-AG is an endocannabinoid that activates the cannabinoid CB_1 receptor. It is involved in a wide array of (patho)physiological functions, such as emotion, cognition, energy balance, pain sensation and neuroinflammation. In this review, we describe the biosynthetic and metabolic pathways of 2-AG and how chemical and genetic perturbation of these pathways has led to insight in the biological role of this signaling lipid. Finally, we discuss the potential therapeutic benefits of modulating 2-AG levels in the brain.

1. Introduction

2-Arachidonoylglycerol (2-AG) is one of the most extensively studied monoacylglycerols. It acts as an important signal and as an intermediate in lipid metabolism [1,2]. It was first isolated from canine gut and identified as an endogenous ligand for the cannabinoid receptors in 1995[3,4]. 2-AG behaves as a full agonist for the cannabinoid CB_1 and CB_2 receptors [5–8], which are also modulated by Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of the plant *Cannabis sativa* [9]. The CB_1 receptor is expressed throughout the body, but it is most extensively studied in the central nervous system (CNS), where it is the most highly expressed G protein-coupled receptor (GPCR) [10,11]. While CB_2 receptor appears to be expressed in the CNS only upon different insults [12], its predominant localization on immune cells is in line with its role in modulating immune responses [13–15]. Several other endogenous biomolecules also interact with CB_1 and CB_2 receptors, including *N*-arachidonoylethanolamine (anandamide; AEA) [16], virodhamine, *N*-arachidonoyldopamine (NADA) and noladin ether (see Pertwee et al. [17] for their *in vitro* binding affinities). 2-AG and AEA are considered to be the most important endocannabinoids. Of note, brain 2-AG levels are ~170 times higher than those of AEA [18]. It is now well established that 2-AG is synthesized "on demand" and acts as a retrograde messenger that inhibits neurotransmitter release at both inhibitory and excitatory synapses, thereby mediating various forms of long- and short-term plasticity [19–22]. Activation of the CB_1 and CB_2 receptors by 2-AG is associated with many physiological processes, including inflammation [23], food intake [24–26], locomotor activity [27,28], learning and memory [29,30], epileptogenesis [31], neuroprotection [32], pain sensation [33], mood [34,35], stress and anxiety [36], addiction [37], and reward [38]. CB_1 receptor signaling is tightly regulated by biosynthetic and catabolic enzymes that control 2-AG levels (Fig. 1). 2-AG and AEA and the CB receptors, together with the biosynthetic and catabolic enzymes of 2-AG, constitute an endogenous signaling system termed "the endocannabinoid system". The aim of this review is to describe the physiological role of 2-AG within the brain with a focus on food intake and metabolism, neuroinflammation, anxiety, addiction and pain. Furthermore, it is discussed how perturbing 2-AG biosynthesis and degradation may facilitate insights into these physiological functions.

2. 2-AG biosynthesis

The biosynthesis of 2-AG can be divided in two main pathways: a signaling pathway that starts from phosphatidylinositol-4,5-bisphosphate (PIP_2), and a metabolic pathway that uses *sn2*-arachidonate-containing triglycerides (Fig. 1). PIP_2 is converted into two important second messengers by phospholipase Cβ (PLCβ) or phospholipase γ (PLCγ): diacylglycerol (DAG) and inositol-1,4,5-triphosphate [18,41,42]. PLCβ uses Ca^{2+} as a cofactor, and acts as a coincidence detector by integrating i) the signals coming from Gq-coupled receptor activation, and ii) the influx of extracellular Ca^{2+} via ionotropic receptors and voltage-gated calcium channels [18,43–47]. The metabolic pathway involves the hydrolysis of triglycerides by hormone-sensitive lipase, carboxyl esterase and other lipases to *sn2*-arachidonoylglycerol

* Corresponding author.
E-mail address: m.van.der.stelt@chem.leidenuniv.nl (M. van der Stelt).
[1] Present address: Institute of Chemical Biology, Department of Chemistry, Imperial College London, London SW7 2AZ, UK.
[2] Equally senior authors.

https://doi.org/10.1016/j.plipres.2018.05.002
Received 3 November 2017; Received in revised form 3 May 2018; Accepted 6 May 2018
Available online 08 May 2018

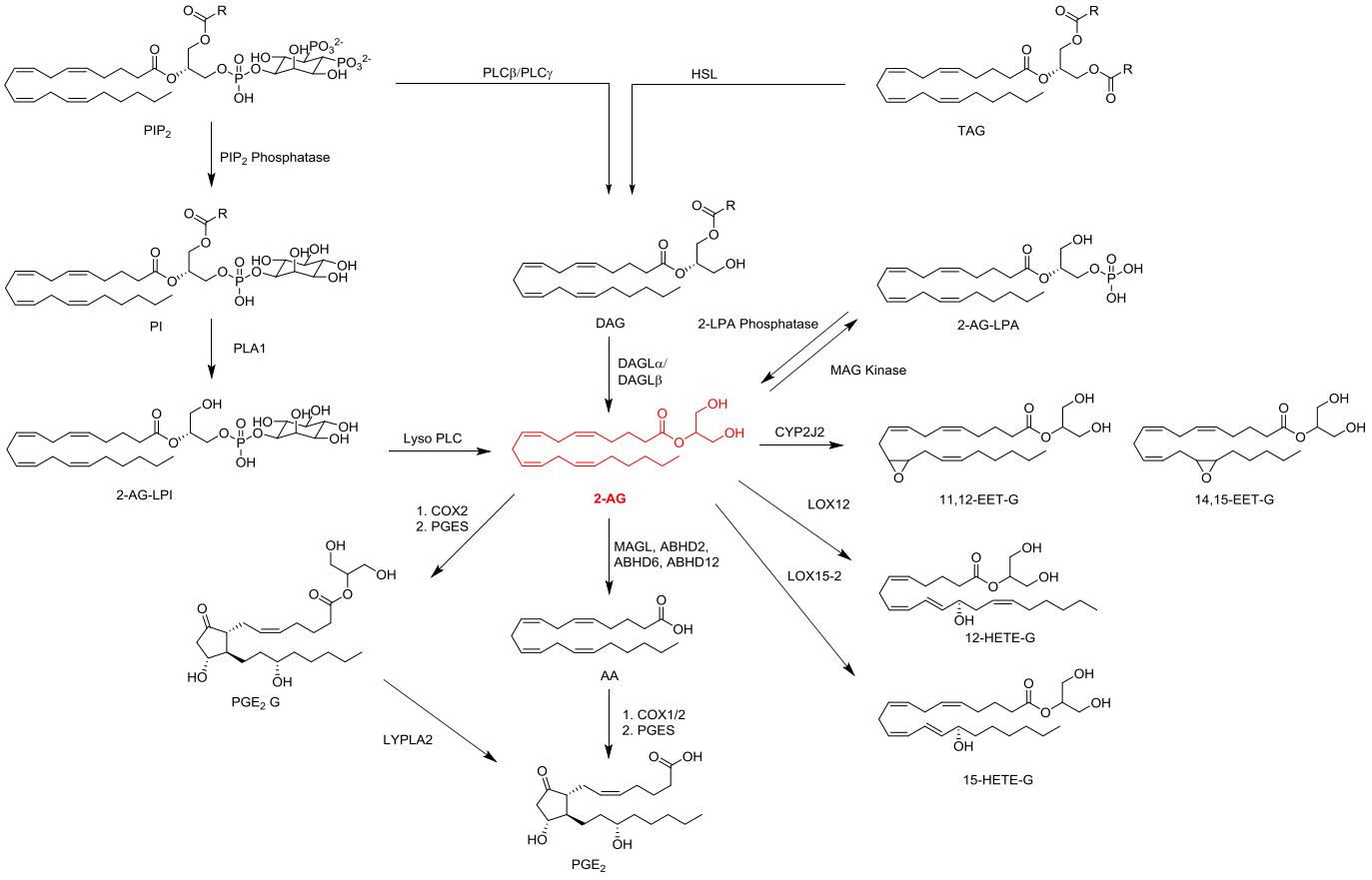


Fig. 1. Overview of the major biosynthetic and metabolic pathways for 2-AG. PLCβ: phospholipase Cβ, PLCγ: phospholipase Cγ, HSL: hormone sensitive lipase, PIP_2: phosphatidylinositol-4,5-bisphosphate, PI: Phosphatidylinositol, TAG: triacylglycerol, DAG: diacylglycerol, PLA1: phospholipase A1, 2-AG-LPI: 2-arachidonoyl lysophosphatidylinositol, Lyso-PLC: lyso-phospholipase C, MAG kinase: monoacylglycerol kinase, 2-LPA phosphatase: 2-lysophospatidic acid phosphatase, 2-AG: 2-arachidonoyl glycerol, 2-AG-LPA: 2-arachidonoylglycerol lysophosphatidic acid, CYP2J2: cytochrome P2J2, LOX-12: lipoxygenase 12, LOX-15-2: lipoxygenase 15-2, MAGL: monoacylglycerol lipase, ABHD2, 6, 12: alpha/beta hydrolase domain containing 2, 6, 12, COX1/2: cyclooxygenase 1/2, LYPLA2: lysophospholipase 2, 11, 12-EET-G: 11, 12-epoxyeicosatrienoic acid glycerol ester, 14, 15-EET-G: 14, 15-epoxyeicosatrienoic acid glycerol ester, PGE_2-G: prostaglandin E_2 glycerol ester, PGE_2: prostaglandin E_2, 12-HETE-G: 12-hydroxyeicosatetraenoic glyceryl ester, 15-HETE-G: 15-hydroxyeicosatetraenoic glyceryl ester. PGES: Prostaglandin E synthase. R = predominantly stearoyl, but it can also represent lipids of different chain length and/or degree of saturation [39,40].

containing diglycerides [18]. At this point the two pathways converge and the diglycerides are further processed by two isoforms of a *sn1*-specific diacylglycerol lipase, diacylglycerol lipase-α and -β (DAGL-α and -β) [48]. The DAG lipases hydrolyze diacylglycerols at the *sn1* position, generating 2-AG and a fatty acid. In addition, DDHD domain-containing protein 2 (DDHD2) has recently been reported to possess also the ability to hydrolyze DAG [49]. Partially purified DDHD2 from rat brain, and rat DDHD2 expressed in Chinese hamster ovary (CHO) cells exhibited DAGL activity *in vitro*. However, Inloes et al. generated a DDHD2 KO mouse, which revealed that DDHD2 is a principal triglyceride lipase *in vivo* [50]. Thus, further research is required to establish the physiological relevance of this pathway for effective 2-AG biosynthesis in the brain.

2-AG can also be generated via an alternative pathway by PIP_2 phosphatase, followed by hydrolysis of the *sn1*-ester by phospholipase A_1 to generate 2-arachidonoyl-LPI. In a final step, the phosphate is removed by lysophospholipase C (lysoPLC) to form 2-AG [51] In 2002, lysophospatidic acid was found indeed in rat brain. This lipid could be quickly converted to 2-AG by 2-LPA phosphatase [52]. Of note, 2-AG can also be processed to 2-arachidonoyl LPA by monoacylglycerol kinase and converted back to 2-AG by LPA-phosphatase. Although these alternative pathways may provide 2-AG, DAGLs are still considered the most important enzymes for its biosynthesis, on the basis of both selective DAGL inhibitor studies and DAGL-α/-β KO studies [53–55].

2.1. Diacylglycerol lipase α and β

The enzymatic activity that hydrolyses the *sn*-1-acyl chain from 1,2-diglycerides yielding 2-AG in human platelets was first detected by Prescott and Majerus in 1983 [72]. Since then, considerable efforts were undertaken to characterize and isolate the enzyme from bovine brain [73,74], and it was not until 2003 that two enzymes were identified, cloned and designated as DAGL-α and -β [48]. The human genes show a high homology compared to those of mouse, 97% for DAGL-α and 79% for DAGL-β. These enzymes structurally differ by the absence of a large C-terminal tail in DAGL-β compared to DAGL-α. Transfection of the DAGL-α and DAGL-β genes in COS cells resulted in enzymes with a molecular weight of ~120 kD for DAGL-α and ~70 kD for DAGL-β. Molecular characterization of these two enzymes revealed that they are plasma membrane-bound serine hydrolases specific for hydrolysis of an acyl chain at the *sn1*-position of diacylglycerols [48].

The C-terminal tail of DAGL-α plays an important regulatory function. Its phosphorylation by calcium/calmodulin-dependent protein kinase II (CaMKII) at Ser^{782} and Ser^{808} significantly reduces enzyme activity [75]. The C-terminal tail of DAGL-α contains also a consensus motif (PPxxF) for binding with the coiled coil domain of homer proteins. Homers are adaptor proteins that interact with many different proteins, including the metabotropic glutamate receptor. An interaction between homer-1b and homer-2 with DAGL-α was shown to be important for DAGL-α localization at the plasma membrane, but is not

required for its catalytic activity [76]. Moreover, the surface expression of DAGL-α is dynamic, undergoing endocytosis and recycling back to the postsynaptic membrane [77]. DAGL-α cycling between the cell surface and intracellular endosomal compartments is regulated by protein kinase C (PKC). Rapid degradation and replenishment of DAGL-α upon inhibition with covalent inhibitor DH376 indicated a short protein half-life [39]. Taken together, these observations indicate that activity, expression and localization of DAGL-α enables a tight spatio-temporal control on 2-AG synthesis and release.

Two landmark studies have shown the contribution of DAGL-α and DAGL-β to the *in vivo* biosynthesis of 2-AG. Congenital deletion of DAGL-α or -β reduced 2-AG levels in the brain by 80% and 50%, respectively [53,54]. In the liver of DAGL-α KO mice a 50% reduction in 2-AG was observed, whereas in DAGL-β KO mice it was almost completely abolished (> 90% reduction), revealing a tissue specific contribution of each enzyme. DAGL-α is highly expressed in neurons and is found at (peri)post-synaptic sites, while DAGL-β is relatively more active in microglia [78–82]. 2-AG acts as a retrograde neurotransmitter. Stimulation of 2-AG release by depolarization of postsynaptic neurons, activation of $G_{q/11}$-coupled receptors and a combination of $G_{q/11}$-coupled receptor activation and Ca^{2+} elevation suppresses synaptic transmission at both excitatory and inhibitory synapses, that is in depolarization-induced suppression of inhibition(DSI) or excitation DSE), respectively. This effect was absent in DAGL-α KO brain slices, but DAGL-β knockout brain slices maintained retrograde inhibition of synaptic transmission [53,54].

Recently multiple, highly selective pharmacological tools have been developed to modulate DAGL activity, but subtype selective inhibitors for each DAGL isoform have not been reported yet. See Kohnz et al. [83] and Janssen et al. [84] for recent reviews on DAGL inhibitors. Dual DAGL-α/β inhibitors used in parallel with DAGL-α and DAGL-β knockout mice have contributed to our understanding of the physiological role of 2-AG in health and disease models, such as synaptic transmission, neuroinflammation [39], anxiety [85] and food intake [86]. For example, Baggelaar et al. reported the highly selective dual DAGL-α/β inhibitor LEI105[58] and Ogasawara et al. disclosed the first CNS active DAGL inhibitors DO34 and DH376 (Table 1) [39]. These inhibitors were instrumental to unequivocally establish that 2-AG production is 'on demand' during various forms of short-term synaptic plasticity in hippocampal and cerebellar slices [39,58,87]. Lipid analysis revealed an extensive rewiring of lipid metabolic pathways in the brain upon inactivation of DAGLs by DO34 and DH376. Not only levels of *sn1*-stearyl-2-arachidonoyl-glycerol, but also levels of *sn1*-palmitoyl-2-arachidonoyl-glycerol, *sn1*-palmitoyl-2-stearyl-glycerol, *sn1*-oleyl-2-stearyl-glycerol were elevated in DAGL-α KO and in inhibitor-treated mouse brains. Concomitantly, 2-AG and other monoacylglycerols, i.e. palmitoyl-glycerol, oleoyl glycerol and docosahexaenoyl-glycerol, were reduced. Interestingly, these other monoacylglycerols are important signaling lipids in their own right, which should be taken into account when analyzing the physiological effect of disrupting DAGL function.

3. 2-AG degradation

2-AG is metabolized by multiple enzymes (Fig. 1), all of which lead to a decrease in 2-AG levels and signaling. The predominant pathway for 2-AG metabolism is the hydrolysis of the ester bond into arachidonic acid (AA) and glycerol. Blankman *et al.* showed that MAGL is responsible for ~85% of 2-AG hydrolysis in mouse brain, whereas α,β-hydrolase domain-containing protein 12 (ABHD12) and ABHD6 account for ~9% and ~4%, respectively [88]. Also cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CytP450) enzymes have been reported to use 2-AG as a substrate. Recently, ABHD2 has been reported to hydrolyze 2-AG in spermatozoa. This enzyme has a relatively high expression in the brain compared to other tissues. These metabolic enzymes are described in more detail below.

3.1. Monoacylglycerol lipase (MAGL)

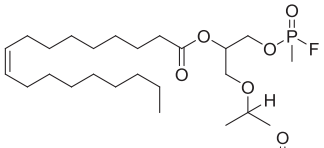
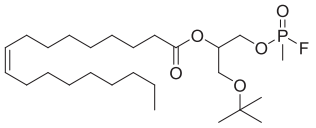
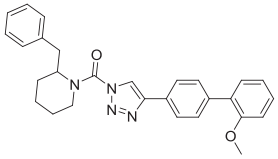
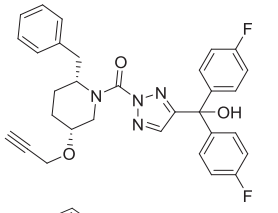
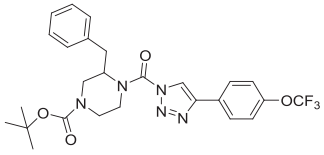
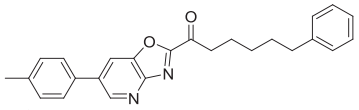
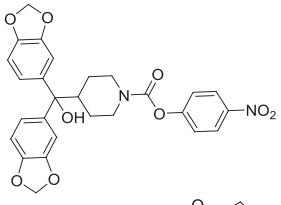
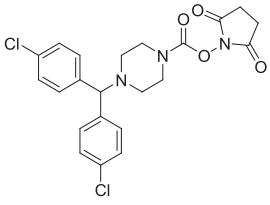
In the early sixties, it was demonstrated that two distinct proteins from lipoprotein lipase were involved in the hydrolysis of triacylglycerol in adipose tissue of both rats and rabbits. Hormone sensitive lipase (HSL) was shown to convert triacylglycerol to monoacylglycerols, and subsequently a dedicated lipase hydrolyzed monoacylglycerols to free fatty acids and glycerol [89]. Partial purification of the monoacylglycerol lipase activity convincingly showed its preference for monoacylglycerols over diacylglycerols and triacylglycerols [90]. It took until 1997 before a specific monoacylglycerol lipase (MAGL) was cloned [91]. MAGL consists of two tissue specific isoforms with a molecular weights of 33 kD and 36 kD, which could reflect different splice variants [91–94]. It is an ubiquitously expressed serine hydrolase containing the typical GXSXG motif with Ser-122, Asp-239 and His-269 forming the catalytic triad [61,64]. The enzyme associates with membranes and its active site resides in the cytosol. MAGL has a broad substrate specificity and hydrolyses monoacylglycerols with different chain lengths and degrees of unsaturation, including 2-palmitoylglycerol, 2-stearoylglycerol and 2-oleoylglycerol [95–97]. Hydrogen peroxide-mediated MAGL sulfenylation at C-201 and C-208 reduces enzyme activity [98].

The interest in MAGL was boosted when the enzyme was linked to the endocannabinoid system. The subsequent development of MAGL KO mice [59,60,83,94,99] and selective, *in vivo* active MAGL inhibitors, such as JZL184 and MJN110 (Table 1), have significantly advanced our understanding of the role of MAGL *in vivo* [59,100–103]. The tissue specific role of MAGL was investigated using the MAGL inhibitor JZL184. The levels of 2-AG were increased 8-fold in the brain, whereas in peripheral tissues such as liver, kidney, spleen, brown adipose tissue (BAT) and heart their increase was less evident. No significant changes were observed in the testis, lung and white adipose tissue (WAT) [94]. Interestingly, C16:0 and C18:1 MAGs were increased in peripheral tissues to a larger extent than in the brain, suggesting a more general metabolic function of MAGL in peripheral tissues [94,102]. MAGL has a relatively high expression in neurons compared to astrocytes and microglia. In contrast to the major 2-AG biosynthetic enzyme DAGL-α, MAGL is positioned presynaptically along with CB_1 receptor. Evidence for the role of MAGL in retrograde 2-AG signaling was shown in electrophysiology experiments in cultured neurons and brain slices, where both MAGL inhibitors and genetic deletion of MAGL enhanced DSI/DSE [104–111].

Chronic disruption of MAGL activity severely decreased the ability of mouse brain to hydrolyze 2-AG, leading to a > 10 fold increase of 2-AG in the brain. Interestingly, a milder but still significant increase of 2-AG levels was observed in thymus, spleen and liver of MAGL KO mice [102]. Noteworthy, levels of other monoacylglycerols were also increased *in vivo*. Tonic elevation of 2-AG levels by MAGL knockout in the brain causes adaptations in CB_1 receptor activity. MAGL knockout mice exhibited impaired endocannabinoid-dependent synaptic plasticity, physical dependence and desensitization of brain CB_1 receptors [101]. Repeated administration of the selective MAGL inhibitor JZL184 mirrored the observations in the knockout mice and caused CB_1 receptor desensitization, tolerance to CB_1 receptor agonists and down regulation of CB_1 receptors [101]. This evidence points to a major role for MAGL in regulating bulk 2-AG levels, and raises the question of whether a therapeutic window to elevate 2-AG levels can be achieved without desensitizing CB_1 signaling. Several studies have addressed this question and investigated the effect of partial blockade of MAGL using low doses of JZL184 [112–115]. Chronic JZL184 dosing for ~7 days up to 8 mg/kg per day did not lead to CB_1 receptor desensitization or behavioral tolerance, while chronically treated animals with 16 mg/kg JZL184 per day did show tolerance [114,115]. Thus, it appears possible to elevate 2-AG levels without desensitizing CB_1 receptor.

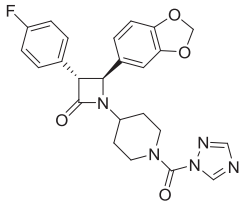
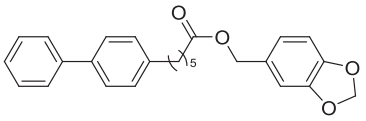
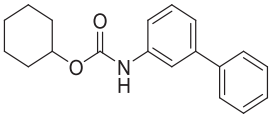
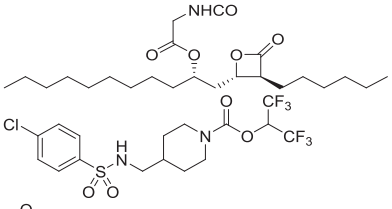
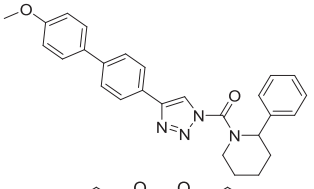
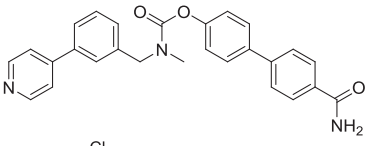
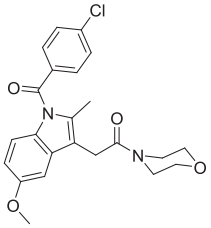
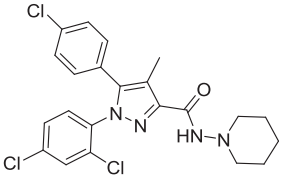
Noteworthy, the content of AA, the product of 2-AG hydrolysis, dropped significantly in the brain devoid of MAGL and DAGL activity

Table 1
Inhibitors and ligands discussed throughout this review.

Enzyme	Struucture	Name	Chemotype	Off target(s)
DAGL		O-7460	Fluoro phosphonates	KIAA1363[56]
		O-5596	Fluoro phosphonates	None reported [57]
		KT172	1,2,3-Triazole ureas	ABHD6[55]
		DH376	1,2,3-Triazole ureas	ABHD6[39] CES1C LIPE BCHE
		DO034	1,2,3-Triazole ureas	DAGL [39] CES1C PLA2G7 ABHD2 PAFAH2
		LEI105	a-Keto heterocycles	None reported [58]
MAGL		JZL184	carbamates	FAAH [59]
		MJN110	carbamates	ABHD6 (minor) [60]

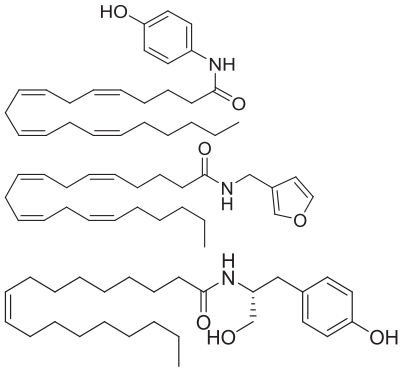
(*continued on next page*)

Table 1 (*continued*)

Enzyme	Struucture	Name	Chemotype	Off target(s)
MAGL		Compound 4a[a]	1,2,4-triazole urea	LYPLA1[61] LYPLA2
		Compound 21[b]	Biphenyl-ester	FAAH (minor) [62]
		URB602	carbamate	FAAH [63]
		OMDM169	β-lactone	FAAH [64] DAGL-α
		SAR127303	carbamate	ABHD6[29]
ABHD6		KT195	1,2,3-Triazole urea	PLA2G15[55]
		WWL70	carbamate	None reported [65]
COX-2		LM-4131	Indomethacin Morpholinylamide	None reported [66]
CB1		Rimonabant	pyrazole-3-carboxamide	ABHD12[67] TRPV3 TRPA1 (minor) TRPM8 (minor) MC3 MT1

(*continued on next page*)

Table 1 (*continued*)

Enzyme	Struucture	Name	Chemotype	Off target(s)
Endocannabinoid transport		AM404	arachidonoylamide	CB_1[68] VR1 vanilloid receptor [69]
		UCM707	arachidonoylamide	CB_2[70] CB_1 (minor)
		OMDM-2	oleylamide	CB_1 (minor) [71]

[a] Compound 21: Benzo [d][1,3]dioxol-5-ylmethyl 6-([1,1'-biphenyl]-4-yl)hexanoate, named compound **4a** in Brindisi et al. [61].

[b] Compound 4a: trans-(1-(1-(1H-1,2,4-Triazole-1-carbonyl)piperidin-4-yl)-4-benzo[d][1,3]dioxol-5-yl)-3-(4-fluorophenyl)azetidin-2-one, named compound **21** in Hernandez-Torres et al. [62].

[39,101,102]. This indicates that the DAGL-MAGL axis is responsible to a large extent for the free AA pool in the brain. Consequently, the activity of MAGL and DAGL affected levels of pro-inflammatory prostaglandins PGE_2 and PGD_2, which are oxidative metabolites of AA, and points to DAGL-MAGL axis as a key pathway for the regulation of neuro-inflammatory responses [39,116].

3.2. α,β-Hydrolase domain-containing 6 protein (ABHD6)

ABHD6 is part of a superfamily of proteins having an α,β-hydrolase fold [117]. It is a membrane-bound protein consisting of 337 amino acids and has a mass of 38 kD [88]. It is a serine hydrolase that contains a catalytic triad with the typical GXSXG motif surrounding the catalytic Ser-148; the other two members of the catalytic triad are Asp-278 and His-306 [118]. mRNA expression analysis in mice showed that ABHD6 is ubiquitous in the body with relatively high expression in BAT and the brain [119]. In the human cortex mRNA expression of ABHD6 steadily increased from neonatal age until adulthood [120]. It is postulated that ABHD6 is membrane bound, with its active site facing the cytosol. ABHD6 was responsible for ~4% of the total 2-AG hydrolysis in mouse brain homogenates [88]. Several selective inhibitors (e.g. KT195 and WWL70 (Table 1)) have been developed to study ABHD6 function [65,121–123]. Endogenous ABHD6 was responsible for ~50% of 2-AG degradation in microglial BV-2 cells. In Neuro2A cells, 2-AG levels were increased after inhibition with the selective ABHD6 inhibitor KT195 (Table 1) [55]. Both Neuro2A and BV-2 cells lack MAGL activity, therefore ABHD6 is an important player in 2-AG hydrolysis in these cell types. Many studies have focused on the role of ABHD6 in 2-AG metabolism and endocannabinoid signaling in the brain. Electron microscopy and immunofluorescence experiments revealed localization of ABHD6 in postsynaptic dendrites in adult mouse brain, whereas MAGL is predominantly expressed pre-synaptically [124], thus positioning ABHD6 at the site of 2-AG production. Inhibition of ABHD6 in prefrontal cortical brain slices by the selective inhibitor WWL70 (Table 1) could reduce CB_1 receptor-mediated long-term depression (LTD) [124]. DSI or DSE in autaptic hippocampal neurons were not reduced by inhibition of ABHD6 [109,125,126]. It is suggested that ABHD6 is an interesting drug target for moderate elevation of 2-AG levels. For example, inhibition of ABHD6 has anti-inflammatory and neuroprotective effects in a mouse model of traumatic brain injury and multiple sclerosis, and shows antiepileptic activity [127–129]. Interestingly, ABHD6 has recently been reported to negatively regulate surface delivery and synaptic function of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which are ionotropic glutamate receptors mediating synaptic transmission. ABHD6 overexpression in neurons could drastically reduce excitatory neurotransmission mediated by the AMPA receptors independently of its hydrolytic activity, thereby possibly contributing to its anti-epileptic activity [130].

3.3. α,β-Hydrolase domain-containing 12 protein (ABHD12)

ABHD12 belongs to the same α,β-hydrolase fold-containing superfamily as ABHD6 [117]. ABHD12 is a membrane-bound protein with an extracellularly oriented active site [88,131]. It is a 398 amino acid containing enzyme with a molecular weight of 45 kD. It is a serine hydrolase having a lipase motif (GTSMG) and a catalytic triad formed by Ser-246, Asp-333 and His-372 [118]. ABHD12 contributes approximately ~9% of the total 2-AG metabolism in mouse brain homogenates [88]. Loss-of-function mutations in the *ABHD12* gene cause the human neurodegenerative disorder PHARC (polyneuropathy, hearing loss, ataxia, retinosis pigmentosa, and cataract) [132]. ABHD12 transcripts are highly expressed in the brain, with a significant enrichment in microglia [133]. No selective ABHD12 inhibitors are as yet available, but the generation of transgenic mice with congenital deletion of ABHD12 has greatly facilitated our understanding of its biological role [134]. ABHD12 knockout mice showed PHARC-like symptoms such as hearing disruptions, muscle weakness and ataxia. Importantly, these mice showed high elevations in lysophosphatidylserines (LPS), whereas the 2-AG hydrolyzing capacity of ABHD12 knockout mice was not significantly different compared to wild-type counterparts. In line with this, ABHD12 overexpression did not affect DSE [109]. However, after incubation with a MAGL inhibitor, a significant reduction in residual 2-AG metabolism was observed in the ABHD12 KO *versus* wild-type mice. This suggests that ABHD12 plays a more specialized role and might be recruited when there is insufficient 2-AG hydrolyzing capacity. However, there are no data to support the notion that endogenous ABHD12 hydrolyses 2-AG *in vivo*. Identification of selective inhibitors that allow spatiotemporal control of ABHD12 activity will be a crucial tool to elucidate the actual role of this enzyme in 2-AG metabolism.

3.4. α,β-Hydrolase domain-containing protein 2 (ABHD2)

ABHD2 is another member of the α,β-hydrolase fold-containing superfamily. The enzyme is a serine hydrolase with a classical Ser-207, His-376, Asp-345 catalytic triad. ABHD2 is predicted to be a single pass type-II membrane protein. The expression of the enzyme is highest in lung, brain and adrenal glands [119]. Knockdown of ABHD2 using

antisense oligonucleotides and gene trapping techniques was used to support an essential role of 2-AG in Hepatitis B virus propagation [135]. ABHD2 appears to play an important role in chronic diseases involved in monocyte/macrophage recruitment, such as atherosclerosis and emphysema [136,137]. It was not until recently that ABHD2 was linked to 2-AG metabolism [138]. The enzyme is highly expressed in spermatozoa and acts as a progesterone-dependent hydrolase. Activation of ABHD2 reduces inhibition of sperm calcium channel CatSper by 2-AG, which leads to calcium influx and stimulates sperm activation. No inhibitors for ABHD2 have been reported to date, and the actual contribution of ABHD2 to 2-AG metabolism in the brain requires further investigation. The cross-talk between progesterone and ABHD2-dependent endocannabinoid metabolism appears of major interest, and clearly extends previous data on the ability of progesterone to control anandamide-hydrolase FAAH expression in human lymphocytes [139], of estrogen to control FAAH expression in mouse Sertoli cells [140], of cholesterol to modulate the CB_1 cannabinoid receptor at a distinct CRAC sequence [141], and of pregnenolone to control CB_1 specific signaling by acting at a specific amino acid residue [142]. Overall, this cross-talk brings about a type of regulation whereby 2-AG is continuously produced to block CatSper, unless progesterone stimulates its hydrolysis by ABHD2 [138]. Therefore, the widely accepted dogma of the "on demand" synthesis of 2-AG, that suggests that this compound acts only upon stimulus-dependent release from phospholipid precursors, might not hold true in all tissues extending previous observations on AEA [143].

3.5. Cyclooxygenase-2 (COX-2)

Cyclooxygenases play an important role in inflammation and exist in two isoforms: cyclooxygenase-1 and -2 (COX-1 and COX-2). The expression of COX-2 is increased in inflammatory processes and is essential for the oxidation of AA to prostaglandins [144]. In contrast to the constitutively expressed COX-1, which has a strong preference for substrates with a free carboxylate group, COX-2 metabolizes both AA and 2-AG with similar K_{cat} and K_m [145,146]. Several papers suggest that COX-2 plays a role in terminating 2-AG signaling in the CNS. For instance, Kim and Alger showed that inhibition of COX-2 prolonged DSI in hippocampal slice preparations [147]. A later study in cultured hippocampal neurons showed that the duration of DSI in a subpopulation of interneurons was influenced by both MAGL and COX-2 activity [125]. Substrate-selective inhibitors for COX-2 have been developed, which selectively block the oxidation of 2-AG and AEA but not of AA [66,148]. The substrate selective inhibitor LM-4131 (Table 1) could increase 2-AG levels in RAW264.7 cells and intraperitoneal (IP) injection of this inhibitor led to an *in vivo* increase of 2-AG levels in the brain [66].

COX-2-mediated oxidation of 2-AG produces the prostaglandin glyceryl ester PGE_2-G (Fig. 1). Most of the effects of substrate selective blockade of COX-2 appear to be caused by changes in PGE_2-G concentrations rather than to be mediated by the minor alterations in 2-AG levels [66]. PGE_2-G is a multifunctional signaling molecule that plays a role in pain, immunomodulation, and synaptic plasticity [149–151]. The receptor for PGE_2-G has not been identified yet, but the actions of PGE_2-G are mediated through extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), IP_3, and NF-κβ [151]. In contrast to the anti-nociceptive and anti-inflammatory role of 2-AG, the COX-2 metabolite PGE_2-G produces hyperalgesia and enhances inflammation-induced neurodegeneration [149,151,152]. Of note is that 2-AG suppresses elevation of COX-2 expression via a CB_1 receptor-mediated mechanism involving PPARγ and the MAPK/NFκ-B signaling pathway [153,154]. PGE_2-G can be further processed by lysophospholipase A_2 ($LYPLA_2$) to generate PGE_2 [155].

3.6. Lipoxygenase

Lipoxygenases are a family of non-heme iron containing dioxygenases that catalyze the oxidation of polyunsaturated fatty acids with one or more (1*Z*,4*Z*)-pentadiene moieties [156]. This oxidation generates the corresponding (1*S*,2*E*,4*Z*)-hydroperoxides. Moody et al. showed that 12-lipoxygenase (12-LOX) oxygenates glycerol esters towards 12-hydroxyeicosatetraenoic (12-HETE) glyceryl ester [157]. Kozak et al. screened a panel of 6 lipoxygenases for their 2-AG oxygenating properties, and found that 15-LOX-1 and 15-LOX-2 also catalyze the oxygenation of 2-AG towards 15-HETE-glyceryl ester [158]. Moreover, among a series of structurally related arachidonoyl esters, 2-AG was the optimal substrate for 15-LOX oxygenation. 2-AG treatment of COS-7 cell transfected with 15-LOX led to the biosynthesis and excretion of 15-HETE-G. Van der Stelt *et. al.* investigated the affinity of LOX metabolites of 2-AG for the cannabinoid receptors, and found that 15-HETE-1-AG has moderate affinity for CB_2 but not for CB_1 receptor [159]. However, to date *in vivo* conversion of 2-AG by 15-LOX or 12-LOX has not been reported yet, therefore the physiological role of this pathway remains to be established.

3.7. Cytochrome P450

Cytochrome P450 (CYP450) is a family of heme-containing oxidative enzymes involved in drug metabolism, that can also use endogenous lipids like 2-AG as substrates [160,161]. Two epoxide regioisomers (2-11, 12- and 2-14, 15-epoxyeicosatrienoic acid glycerol ester (EET-EG)) of CYP450-generated metabolites of 2-AG have been isolated from rat kidney and spleen, whereas 2-14, 15-EET-EG was also detected in the brain [162]. These metabolites have a higher binding affinity for CB_1 and CB_2 receptors compared to 2-AG, whereas the corresponding oxidized metabolites from AA (11,12-EET and 14,15-EET) do not display any CB_1 or CB_2 receptor affinity. Incubation of 2-AG with the predominant CYP450 in the heart (CYP2J2 epoxygenase) produces 2-11, 12- and 2-14, 15-EET-EG *in vitro* [163]. In addition, heart microsomes derived from bovine and porcine tissues also produced 2-11, 12- and 2-14, 15-EET-EG. Yet, the physiological relevance of these CYP450 metabolites remains to be elucidated.

4. 2-AG transport

In contrast to polar neurotransmitters that are stored in vesicles, AEA and 2-AG are neutral lipids that have the intrinsic tendency to associate with membranes. It is poorly understood how 2-AG and AEA move across the synapse and traverse within the cellular interior, and how cellular uptake of 2-AG and AEA is regulated. Possible routes for 2-AG and AEA transmembrane transport are passive diffusion, endocytosis, transporter proteins, or a combination of these mechanisms. The transport of AEA has been subject of intensive research, but the mechanisms of uptake and transport of this endocannabinoid are still under debate (for reviews see Niculussi & Gertsch [164], and Fowler [165,166]). Fewer studies have addressed the transport process of 2-AG, because its metabolism complicates the analysis of the transport processes. Ben Shabat and colleagues found the first indication of cellular 2-AG uptake [167]. Co-incubation of 2-AG with 2-lineoyl-glycerol reduced 2-AG depletion from media, while 2-palmitoyl-glycerol had no effect [167]. 2-AG accumulation in rat basophilic RBL-2H3 and mouse neuroblastoma N18TG2 cells was also observed by Di Marzo et al. [168]. Saturable 2-AG uptake by human astrocytoma cells, with a Michaelis-Menten constant (K_m) of 0.7 ± 0.1 μM and a V_{max} of 28 ± 6 (pmol/min)/mg, indicated the existence of a specific transporter protein [169]. In addition, 2-AG uptake could be inhibited by AEA and the putative AEA transport inhibitor AM404 (Table 1) [170,171]. Recently, it was found that UCM707 and OMDM-2 (Table 1) could also inhibit 2-AG and AEA uptake with equal potency. Thus, a common carrier mechanism was proposed for both AEA and 2-AG [172]. Importantly, not

only the uptake could be blocked, but also the release of 2-AG, which is suggestive of a bidirectional trafficking of 2-AG via its putative transporter. Such an in and out transport through the plasma membrane has been indeed documented for AEA [173,174]. Identification, and functional characterization of the putative transporter protein(s) will be highly important for a better understanding of 2-AG storage, release and transport. Noteworthy, evidence for intracellular 2-AG transport was recently reported. A fluorescence polarization assay with a NBD-stearate probe showed that 2-AG (as well as AEA) bind to the cytosolic carrier protein fatty acid binding protein (FABP) 5 [175]. Further evidence for this binding was provided in a co-crystalization study of FABP5 and 2-AG [176]. It would be interesting to see to what degree FABP5 regulates the physiological role of 2-AG. Of note, also for the other major endocannabinoid AEA different intracellular transporters (e.g., different FABPs and heat shock protein 70) have been identified that ferry it to the correct final destination [143], as well as extracellular transporters like microvesicles that ferry it through the synaptic cleft [177].

5. Non-CB_1/CB_2 receptors targeted by 2-AG

Interestingly, several reports indicate that 2-AG may also produce physiological effects independently of CB_1 and CB_2 receptors, for instance by binding to and activating $GABA_A$ [178], PPARγ [179], Adenosine A_3 [180], TRPV1 [181], and GPR55 [182]. For example, it has been shown that 2-AG potentiates $GABA_A$ receptor function at low GABA concentrations, which leads to inhibition of motility [178]. It was also shown that 2-AG binds at the M4 transmembrane region of the β2 subunit, and the observed effect was still present in CB_1/CB_2 double knockout mice [178]. These interactions with $GABA_A$ receptors can have important implications on studies that interrogate 2-AG role on sedation and locomotion [178]. In addition, 2-AG has been reported to reduce interleukin (IL)2 expression via a PPAR-γ in splenocytes from CB_1/CB_2 null mice [179,183]. However, it is not completely clear if this interaction is mediated by 2-AG itself or through COX2-generated metabolites thereof [183].

Adenosine is an orthosteric endogenous ligand for the adenosine A_3 receptor. This GPCR plays an important role in modulation of inflammation [184]. 2-AG acts as a negative allosteric modulator of human adenosine A_3 receptor binding. Therefore, adenosine A_3 receptors can trigger potentially alternative pathways for 2-AG to mediate inflammatory responses [185]. Moreover, 2-AG has also been reported to act as a ligand for the TRPV1 receptor, which is an outwardly rectifying Ca^{2+} permeable nonselective cation channel. The latter receptor is activated by stimuli such as heat (T > 42°C) and low extracellular pH, and also by endogenous ligands including lysophosphatidic acid and AEA. TRPV1 plays an important role in the regulation of core body temperature and inflammatory pain [186]. Therefore, TRPV1 is an important target to consider when studying the relation between 2-AG and pain sensation [181,187–189]. Rydberg et al. showed that 2-AG has a high affinity for GPR55 *in vitro* [182]. The latter receptor has been linked to energy balance and is a potential drug target for various cancer types [190]. Of note, not only 2-AG but also other endocannabinoids show affinity for GPR55, including virodhamine, AEA, oleamide and noladin ether. Together, these studies indicate that 2-AG has a wide variety of targets distinct from the classical cannabinoid receptors (Table 2). To a large extent, the physiological relevance of these non-CB_1/CB_2 receptors remains yet to be established *in vivo*, and further research is deemed necessary to reveal the actual physiological effects governed by 2-AG through them.

6. Physiological effects of 2-AG within the brain

During the last decade multiple lines of research have allowed to gain insight in the physiological role of 2-AG. In the following section we will discuss distinct pharmacological tools (Table 1) used to manipulate 2-AG levels, as well as genetic manipulations of its target receptors and metabolic enzymes that have uncovered the biological role of 2-AG and its metabolites in brain function.

6.1. Food intake and metabolism

The intimate link between the endocannabinoid system and energy balance is well-established [201]. CB_1 receptor knockout mice display a lean phenotype, reduced adiposity and feeding efficiency. When fed a high fat diet, they display decreased triglyceride, plasma insulin, cholesterol, and leptin levels, while having increased adiponectin levels compared to their wild-type littermates [202,203]. This phenotype can be mirrored by treatment of wild-type animals with the CB_1 inverse agonist rimonabant (Table 1) [204]. Importantly, rimonabant was approved on the European market in 2006 as a drug for the treatment of obesity. However, it was soon withdrawn in 2008, due to serious psychiatric side effects, in particular depression.

Several lines of evidence indicate that 2-AG is, to a large extent, responsible for CB_1 mediated regulation of food intake and energy metabolism. Fasting increased levels of 2-AG in limbic forebrain and hypothalamus. Additionally, bilateral injection of 2-AG into the nucleus accumbens stimulated increased feeding, and pretreatment with rimonabant could reduce the effect elicited by 2-AG infusion [205]. DAGL-α knockout mice showed a similar phenotype to CB_1 knockout mice with decreased body weight and low triglycerides, cholesterol and insulin levels [206]. Such a phenotype was not observed in DAGL-β knockout mice, pointing to 2-AG biosynthesis by DAGL-α as the underlying mechanism. Acute disruption of DAGL activity by (i.p.) administration of O-7460, a fluorophosphonate-based DAGL inhibitor, dose dependently inhibited intake of high fat diet in mice [56]. This is in line with a previous report where the DAGL inhibitor O-5596 reduced the intake of palatable food in these animals [57].

Jung et al. provided further evidence for the involvement of 2-AG in metabolism [207]. Upregulation of 2-AG hydrolysis by overexpression of MAGL in the mouse forebrain led to a 50% decrease of 2-AG levels in this brain region, but did not affect AEA or AA levels. These mice were lean, hyperphagic, resistant to diet-induced obesity, hyperthermic and hypersensitive to β_3-adrengenic-stimulated thermogenesis. MAGL inhibition with JZL184 showed that restoring 2-AG signaling at CB_1 receptors normalized β_3-adrengenic-dependent thermogenesis, suggesting that such a signaling pathway helps to conserve body energy by sparing heat production.

Despite rimonabant retraction from the market, enzymes responsible for the biosynthesis of 2-AG remain interesting drug targets for the treatment of the metabolic syndrome. Their partial inhibition, subtype selectivity and/or the ability to keep other CB_1 signaling pathways (e.g. AEA) intact, may prevent psychiatric side-effects.

6.2. Neuro-inflammation

2-AG plays a central role in the regulation of multiple neuro-inflammatory processes in the brain. Previously, phospholipase A_2 (PLA_2) was considered the primary source of AA for COX-mediated biosynthesis of prostaglandins, but this view has significantly changed in recent years. Nomura et al. showed that hydrolysis of 2-AG by MAGL provides the major pool of AA for the generation of neuro-inflammatory eicosanoids in specific tissues, such as brain, liver and lungs [116]. Consistently, chemical inhibition or genetic disruption of MAGL activity lowered lipopolysaccharide (LPS)-induced pro-inflammatory eicosanoid production in the brain, such as PGE_2, PGD_2, PGF_2, and thromboxane B_2 (TXB_2), via a CB_1 and CB_2 receptor-independent mechanism. In addition, abolishment of MAGL activity did not affect basal cytokine levels, but almost completely blocked LPS-induced elevation of interleukin-1α (IL-1α), IL-1β, IL-6 and TNF-α. Specific knockdown of MAGL in astrocytes moderately elevated 2-AG levels and did not cause CB_1 receptor desensitization nor any behavioral effect; yet, it decreased

Table 2
2-AG-interacting proteins.

	Receptor	Ligand	K_i, IC_{50} or EC_{50}	Additional ligands	physiological effect	Reference[a]
Receptor interaction	CB_1	2-AG	K_i = 34.6–472 nM[b]	AEA, Virodhamine, Noladin ether [191], NADA [192], oleamide [193], DTEA[c] [194], HLEA[d] [194], EPEA[e] [195], DHEA[f] [195], Hemopressin[196]	Anxiety, pain, metabolism, addiction, inflammation	[3], [167]
	CB_2	2-AG	K_i = 1400 nM[b]	AEA, Noladin ether [191], NADA [192], DLEA [194], EPEA [195], DHEA [195]	Inflammation	[3]
	TRPV1	2-AG	EC_{50} = 2.5 μM[g]	AEA, NADA [197]	Pain	[181]
	$GABA_A$	2-AG	EC_{50} = 2.1 μM[h]	GABA		[178]
	A_3	2-AG	IC_{50} = 12.6 μM[i]	Adenosine		[180]
	GPR55	2-AG	EC_{50} = 3 nM[j]	Lyso-PI, AEA, noladin ether, virodhamine		[182]
	PPARγ	2-AG	EC_{50} = 10 μM[k]	Prostaglandins	Inflammation	[179]
	Enzyme	substrate	K_m	Additional substrates	physiological effect	Reference
Biosynthesis	DAGL-α	DG (18:0/20:4)	155 μM[l]	DG(18:1/18:1), DG(18:1/18:0), DG (16:0/18:0), DG(16:0/20:4), DG(18:1/20:4).	metabolism, anxiety, inflammation	[40]
	DAGL-β	DG (18:0/20:4)	74 μM[l]	DG(18:1/18:1), DG(18:1/18:0)	Inflammation	[40]
	DDHD2	DG (18:0/20:4)	248 μM[m]	TAG species with different combinations of C16:0, C18:0, C18:1, C20:4, C22:5, C22:6 fatty acyl chains [50] (DG 18:0/18:2), DG (18/22:6).	–	[49,198]
	2-LPA Phosphatase	2-AG-LPA	-	Oleoyl-LPA	–	[52]
	Lyso PI-PLC	2-AG-LPI	-	LysoPC, LysoPS [199]	–	[4,51]
	Enzyme	Metabolite	K_m	Additional substrates	physiological effect	Reference
Metabolism	MAGL	AA	110 μM[n]	MG (18:1), MG(18:2) [102] MAG (16:0), MAG (18:1), MG (18:2), MG (22:6) [101]	Anxiety, pain, addiction, metabolism	[118]
	ABHD6	AA	159 μM[n]	MG(14:0), MG(16:0), MG(18:0), MG(18:1) [200] *in vivo* in BAT ABHD6 KO mice.	Inflammation, metabolism	[118]
	ABHD12	AA	117 μM[n]	LPS(16:0), (18:0), (20:4), (22:4), (22:1), (22:0), (18:0), (18:0) and PS(16:0/18:1), (18:1/18:1), (16:1/20:4), (18:1/20:4), (18:0/20:4), (20:1/20:4), (20:0/20:4), (22:1/20:4), (22:0/20:4), (24:1/20:4) [134]	Inflammation	[118]
	COX2	PGE_2-G	4.4 μM[o]	AA, AEA [66]	Inflammation	[145]
	LOX12	12-HpETE-G	6 μM[p]	AA, AEA, linoleic acid, docohexanoic acid	Inflammation	[157]
	LOX15-2	15-HpETE-G	9 μM[q]	AA, AEA	Inflammation	[158]
	CYP2J2	11,12-EET-G 14,15-EET-G	13.6 μM[r] 32.6 μM[r]	AA, AEA	Inflammation	[160,163]

[a] Reference regarding the K_i, K_m, IC_{50} or EC_{50}.
[b] Range of K_i's measured by ligand displacement assay with [^{3}H]HU-243 in membranes fractions of COS cells transfected with the corresponding receptor [3,167].
[c] DTEA: docosatetraenoylethanolamide.
[d] HLEA: homo-γ-linolenoylethanolamide.
[e] EPEA: eicosapentaenoylethanolamide.
[f] DHEA: docosahexaenoylethanolamide.
[g] Effect on intracellular calcium concentration in TRPV1 expressing HK293 cells, maximum responses were normalized to the 10 μM capsaicin-induced response.
[h] Dose-dependent potentiation of currents elicited by 1 μM GABA.
[i] Ligand displacement assay with [^{125}I] AB MECA in CHO membranes expressing the hA_3 receptor.
[j] GTPγS binding assay.
[k] IC50 competition for fluorescent ligand occupancy the purified PPARγ ligand binding domain.
[l] Membrane of COS cell overexpressing DAGL-α or DAGL-β respectively.
[m] Recombinant rDDHD2.
[n] fluorescent glycerol assay for 2-AG hydrolase activity in HEK293 cells transiently transfected with the corresponding hydrolase.
[o] Oxygenation by purified human COX2 [145].
[p] UV assay for the conjugated diene (leukocyte LOX12) [157]. [q]Hexahistidine-tagged human 15 LOX-2 expressed in Sf-9 cells and purified on Ni-NTA agarose.
[r] Formation kinetics for CYP2J2 nanodisc incubations with 2-arachidonoyl glycerol.

cytokine and prostaglandin levels via a CB_1/CB_2 independent mechanism [208]. This is in line with the anti-inflammatory effects observed when blocking 2-AG biosynthesis. DAGL-α/β inhibitors DO34 and DH376 reduced brain levels of 2-AG, AA and AEA [39]. Pro-inflammatory prostaglandins PGE_2 and PGD_2 and cytokine IL-1β were also reduced in LPS-treated animals. In keeping with these observations, Viader et al. showed that DAGL-β is important for regulation of 2-AG levels in microglia without affecting global 2-AG and prostaglandin levels in the brain [78]. Finally, LPS-induced anapyrexia, that lowers core body temperature, was substantially reduced in DAGL-α and DAGL-β KO as well as in inhibitor-treated mice [78], an observation that extends an independent report showing increased anapyrexia in MAGL inhibitor-treated mice [209]. These data imply an important role for 2-AG in the regulation of body temperature.

In addition to playing a pro-inflammatory role by promoting PGs biosynthesis, 2-AG is also neuroprotective. For example, in 2001 Panikashvili et al. reported neuroprotection by 2-AG in a traumatic brain injury model [210]. After infliction of closed head injury, a strong elevation (up to 10-fold increase after 4h) of mouse brain 2-AG levels was observed. Injection of exogenous 2-AG elicited a neuroprotective effect, which was absent in CB_1 knockout models and after pretreatment with the CB1 receptor antagonist rimonabant. Neuro-inflammation is one of the early neurochemical responses after closed head injury [211], and consistently 2-AG has been shown to inhibit NFκ-B transactivation and to reduce acute expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 via CB_1-dependent signaling [212].

Of note, common neurological disorders like Multiple Sclerosis (MS), Parkinson's disease (PD) and Alzheimer's disease (AD) have a

Table 3
Effect of 2-AG modulation in neuro-inflammation.

Disease	Target	Compound and/or knockout (KO)	2-AG levels	Effect	Species	Model	CB receptor mediated	Ref.
Neuro-inflammation	MAGL	JZL184, KO	↑	Reduction of eicosanoid production	Mouse	LPS treatment	No	[116]
	DAGL	DO34, DH376, KO	↓				No	[39]
	MAGL	JZL184	↑	Exacerbates LPS induced anapyrexia			Yes	[209]
	DAGL	DO34, DH376, KO	↓	Attenuates LPS induced anapyrexia			–	[39]
	DAGL-β	KO	↓				–	[78]
	MAGL	Specific KO in astrocytes	↑	Reduction of PGE_2 andcytokine levels			No	[208]
Parkinson's Disease (PD)	MAGL	JZL184, KO	↑	Neuroprotection (prevented MPTP-induced dopaminergic neuronal loss)		(MPTP) mouse	No	[116]
	MAGL	JZL184	↑			Model of Parkinsonism	No	[216]
Alzheimer's disease (AD)	MAGL	JZL184, KO	↑	Attenuate prostaglandin and β-amyloid (Aβ) levels		$PS1/APP^+$ AD Mouse Model	No	[217]
	MAGL	JZL184, KO	↑	Reduction of neurodegeneration		5XFAD APP transgenic mice, a mouse Model of AD	No	[218]
Multiple sclerosis (MS)	MAGL	Compound 21[a]	↑	Decreasing tissue damage		Autoimmune ence-phalitis	–	[62]
	MAGL	Compound 4a[b]	↑	Amelioration of the course of demyelination		(EAE) mouse model MS	Partially	[61]
Huntington's disease (HD)	DAGL	O-3841	↓	Attenuation malonate-induced GABA and BDNF deficiencies	Rat	Malonate model of HD	–	[219]
	MAGL	JZL184	↑	Exacerbation malonate-induced striatal damage			–	[219]

[a] INH21: Benzo [d][1,3]dioxol-5-ylmethyl 6-([1,1'-biphenyl]-4-yl)hexanoate, named compound **4a** in Brindisi et al. [61].
[b] INH4a: trans-(1-(1-(1H-1,2,4-Triazole-1-carbonyl)piperidin-4-yl)-4-benzo[d][1,3]dioxol-5-yl)-3-(4-fluorophenyl)azetidin-2-one, named compound **21** in Hernandez-Torres et al. [62].

major neuro-inflammatory component [213–215]. Consequently, modulation of MAGL activity has been tested in various animal models of neurodegenerative diseases (Table 3). Nomura et al. used a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD [116], where JZL184 provided neuroprotective effects that were not reversed by the CB receptor antagonists, but were recapitulated by COX inhibition. These findings indicate that the neuroprotective effects were not mediated via CB receptors, but were rather due to reductions in AA and proinflammatory prostaglandins. Later on, Mounsey et al. confirmed this hypothesis [216].

Perturbation of MAGL activity has also been investigated in animal models of AD, such as 5XFAD and $PS1/APP^+$ mice. Genetic and pharmacological inactivation of MAGL suppresses proinflammatory responses and amyloidosis in a $PS1/APP^+$ AD mice [217]. Moreover, in 5XFAD AD mice, inhibition of MAGL reduces production and accumulation of β-amyloid (Aβ) and improves cognitive function [218]. In mice suffering from experimental autoimmune encephalitis (EAE), a mouse model for of MS, inhibition of MAGL activity reduced the severity of the clinical symptoms [61,62]. Additionally, inhibition of DAGL with the fluorophosphonate-based inhibitor O-3841 was neuroprotective in a malonate model of Huntington's disease (HD) in rat brain [219]. In contrast, inhibition of MAGL activity and administration of PGE_2-G worsened malonate-induced injury. Therefore, oxidative metabolites of 2-AG have been suggested to play a role in cytotoxicity ultimately leading to HD.

6.3. Anxiety

Endocannabinoid signaling via CB_1 receptor has a primary role in modulating anxiety and depressive behaviors [36,220]. The effects of CB_1 receptor agonists on anxiety are complex and show biphasic effects, with low agonist doses leading to anxiolytic effects [221,222], and high doses leading to anxiogenic effects [223]. Pharmacological blockade and genetic deletion of the CB_1 receptor has been shown to increase anxiety [221,224–227]. Both AEA and 2-AG play an important role in stress and anxiety. Studies have generally shown a bidirectional effect of stress on endocannabinoid levels, AEA levels decreasing and 2-AG levels increasing compared to controls [228,229]. Interestingly, several studies support the hypothesis that glucocorticoids modulate GABA and glutamate release via enhanced endocannabinoid signaling at the CB_1 receptor [230]. Glucocorticoid hormones that are released as a result of stress recruit 2-AG and promote termination of hypothalamic-pituitary-adrenocortical (HPA) axis activity [231,232]. In line with this, it has been shown that levels of 2-AG are significantly elevated by corticosterone treatment and strain-induced stress [233,234]. Elevation of AEA levels by genetic deletion or pharmacological inhibition of FAAH has shown anxiolytic effects in several independent studies [221,235–237]. More recently, the role of 2-AG in anxiety-like behavior has been studied. Shonesy et al. investigated the role of DAGL-α in anxiety [238], documenting increased anxiety and depressive behavior in DAGL-α KO mice. This effect was reversed by normalization of 2-AG content through pharmacological inhibition of MAGL. Furthermore, anxiolytic effects are observed when 2-AG levels are increased by treatment with JZL184 [59,112,114,239–241]. These data show that 2-AG, rather than its downstream metabolites, reduces anxiety. In line with this hypothesis, Jenniches et al. also observed a similar anxiogenic phenotype in DAGL-α knockout mice [242]. Bluett et al. investigated the role of 2-AG in stress resilience in mice [85], showing that acute systemic 2-AG augmentation promotes it. In contrast, CB_1 receptor blockade or pharmacological inhibition of DAGL by DO34 allowed stress-resilient mice to develop anxiety-like behavior after acute stress exposure. The latter finding demonstrates that acute blockade of DAGL-α also results in anxiogenic behavior. Remarkably, in most of the above studies the anxiolytic effect could be blocked by rimonabant, indicating a likely engagement of CB_1 [59,85].

6.4. Addiction

The endocannabinoid system has been implicated in multiple aspects of addiction [37,243,244]. CB_1 receptor agonists have been shown to alleviate withdrawal symptoms [245,246], while inverse agonists and antagonists reduce nicotine self-administration [244]. Several lines of evidence indicate an important role for 2-AG in addictive behavior and withdrawal. Injection of exogenous 2-AG was moderately effective in reducing the intensity of precipitated withdrawal signs in morphine-dependent mice [246], whereas Ramesh et al. [247] showed that JZL184 could completely prevent naloxone-precipitated morphine withdrawal symptoms, including paw flutters,

diarrhea, jumps, and weight loss. These effects were blocked by rimonabant, suggesting a CB_1 receptor-mediated effect. Importantly, somatic and aversive withdrawal signs in nicotine-dependent mice were reduced by both genetic and pharmacological inactivation of MAGL via a CB_1 receptor mediated mechanism [248].

A study by McReynolds *et al.* suggests that stress-induced relapse to cocaine seeking depends on 2-AG and is reduced by CB_1 receptor antagonism [243,249,250]. In keeping with experiments with CB_1 receptor antagonists [251], reduction of 2-AG levels by inhibition of its biosynthetic enzymes DAGL-α and DAGL-β reduced nicotine self-administration in mice. Release of the inhibitory neurotransmitter GABA was diminished upon chronic nicotine exposure [244,252]. To gain insight into the underlying mechanism, the effect of DAGL inhibition on GABA signaling was studied. The DAGL blocker 1,2,3-triazole urea KT172 rescued GABAergic signaling at dopaminergic neurons in the vental tagmental area [252]. Conversely, increasing 2-AG signaling by blocking MAGL recapitulated the loss of nicotine-induced GABA signaling following chronic nicotine exposure.

6.5. Pain

Cannabinoids have been used for their ability to reduce pain for many centuries [253]. The anti-nociceptive effects appear to be mediated by CB_1 receptors within the CNS [254,255], and via both CB_1 and CB_2 receptors at the periphery [33,256,257]. Stimulation of both CB receptor subtypes by AEA or 2-AG shows analgesic effects. The role of AEA in pain sensation has been well-documented. Elevation of AEA levels by inhibition or genetic ablation of FAAH, and administration of AEA elicit anti-nociceptive effects (see Guindon and Hohmann [258], and Schlosburg *et al.* [259] for extensive reviews).

Elevation of 2-AG has been observed in preclinical models of inflammatory pain [260,261]. Various studies have demonstrated an analgesic effect of local administration of 2-AG [262,263], as well as by increasing endogenous 2-AG levels via MAGL inhibitors in various pain models [264–266], including neuropathic pain [267], peripheral inflammatory pain [115], gastrointestinal pain [268] and chemotherapy-induced neuropathy (Table 4) [269,270]. Anti-nociceptive effects of 2-AG are likely mediated by CB_1 receptors. Long et al. and Kinsey et al. showed that the effect of MAGL inhibition on thermal, noxious chemical, and neuropathic pain sensation were blocked by the CB_1 receptor inverse agonist rimonabant [59,269]. Important to note is that the anti-nociceptive effect was absent or largely reduced in MAGL knockout mice and upon chronic treatment with MAGL inhibitors, an observation that can be explained by CB_1 receptor desensitization and/or downregulation [100,101]. Intracerebroventricular injection of JZL184 produced TRPV1-dependent anti-nociception in the mouse formalin test, which indicates that anti-nociceptive effects are also mediated by vanilloid receptors [189]. Recently, inhibition of DAGL-β was reported to reduce nociception in preclinical models of inflammatory and neuropathic pain, which might be explained by a reduction of pro-inflammatory prostaglandins [271]. Taken together, these data demonstrate an important role for 2-AG in pain sensation via multiple mechanisms, including TRPV1, CB_1, and CB_2 signaling, and as a precursor for prostaglandins that modulate inflammation and pain.

7. Conclusions

CB_1 receptor is the major brain target through which 2-AG exerts its physiological effects, including regulation of food intake and metabolism, neuro-inflammation, addiction, anxiety and pain. In addition to classical CB receptors, 2-AG has shown remarkable affinity for other receptor targets, including $GABA_A$, adenosine A_3, TRPV1, PPARγ and GPR55 receptor. To date it is, however, unknown whether these proteins are involved in physiological effects of 2-AG *in vivo*. Next to its function as a signaling lipid, 2-AG is also a key intermediate of multiple lipid metabolic pathways. A number of 2-AG metabolites have important signaling functions in their own right. 2-AG is a key precursor for the biosynthesis of eicosanoids in the brain, which in turn propagate inflammation [146]. Its involvement in major (patho)physiological processes makes modulation of 2-AG levels an interesting strategy from a therapeutic perspective.

Modulation of 2-AG levels *in vivo*, in particular by blocking MAGL or DAGL activity, triggers multidirectional effects in various (patho)physiological processes (Fig. 2). Reduction of 2-AG levels by blocking its major DAGL-α/β biosynthetic pathway can be beneficial for treatment of the metabolic syndrome, addiction, and neuro-inflammatory disorders, while elevation of 2-AG levels by blocking MAGL, the major pathway releasing AA, reduces neuro-inflammation, anxiety-related behavior, withdrawal symptoms and nociception. However, its prime role in multiple important physiological pathways puts modulation of 2-AG levels at risk of unwanted effects (Fig. 2). The essential role of endocannabinoid signaling in the brain was emphasized by the withdrawal of the CB_1 inverse agonist and anti-obesity drug rimonabant from the European market, because of severe neuropsychiatric side effects.

It is important to note that not all behavioral phenotypes of CB_1 KO mice were mirrored by DAGL-α KO mice, which showed less anxiogenic behavior compared to CB_1 KO mice in open field test and platform tests

Table 4
2-AG modulation in pain.

Modulation	Enzyme	Inhibitor	2-AG	Effect	Species	Model	Receptor	Ref.
Genetic	MAGL	–	↑	No effect compared to wild-type	Mouse	Tail-immersion test for thermal nociception	CB_1	[101]
		–	↑	No effect compared to wild-type	Mouse	Inflammatory complete Freund's adjuvant and neuropathic spinal Nerve ligation pain	CB_1	[100]
Inhibition	MAGL	JZL184	↑	Anti-nociceptive	Mouse	Intraplantar formalin injection	TRPV1	[189]
		URB602	↑	Anti-nociceptive	Rat	Radiant-heat tail-flick test	CB_1	[266]
		JZL184	↑	Anti-nociceptive	Mouse	Tail-immersion test for thermal nociception	CB_1	[272]
		JZL184	↑	Anti-nociceptive	Mouse	Tail-immersion test for thermal nociception	CB_1	[101]
		JZL184	↑	Anti-nociceptive	Rat	Mechanical and cold allodynia (cisplatin induced)	CB_1, CB_2	[267]
		JZL184	↑	Reduction of mechanical allodynia	Mouse	Mechanical allodynia (carrageenan)	CB_1, CB_2	[115]
		JZL184	↑	Reduction of allodynia	Mouse	Chronic constriction injury induced allodynia	CB_1	[270]
		OMDM169	↑	Anti-nociceptive	Mouse	Formalin test	CB_1, CB_2	[64]
		JZL184	↑	Reduction of allodynia	Mouse	Chronic constriction injury induced allodynia	CB_1	[269]
		SAR127303	↑	Anti-nociceptive	Mouse	Phenylbenzoquinone-induced writhing model of acute visceral pain and formalin test of inflammatory pain	CB_1	[29]
		URB602	↑	Anti-nociceptive	Mouse	Plethysmometer and plantar test	CB_2	[273]
	DAGL	KT109	↓	Reversal of LPS-induced allodynia	Mouse	Chronic constriction injury, and paclitaxel model of chemotherapy-induced neuropathic pain	NT	[271]

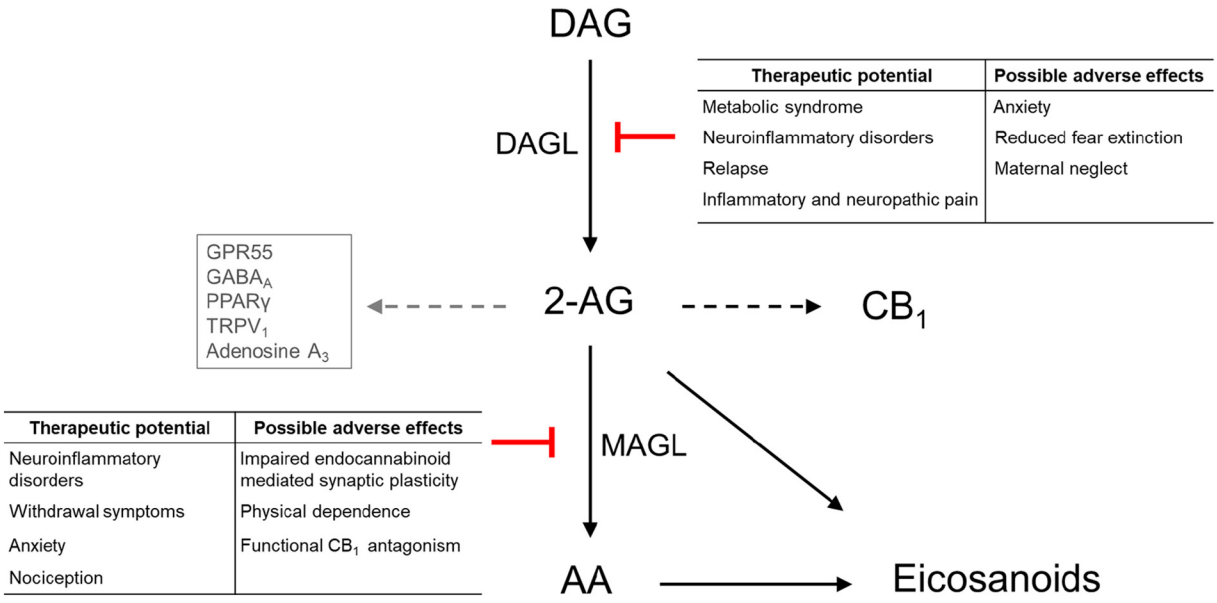


Fig. 2. 2-AG plays a central role in multiple pathophysiological processes. Both elevation and reduction of 2-AG has therapeutic potential. However, untoward neurological effects could pose a serious threat for the use 2-AG modulation for the treatment of human disorders.

[206]. These differences can be partly explained by keeping other CB_1 signaling pathways intact, (e.g., those mediated by AEA) when modulating 2-AG levels. Therefore, selective modulation of 2-AG metabolism can help to dissect the signaling tasks of 2-AG and AEA, and potentially circumvent adverse effects observed by direct blockade of CB_1 receptors. In addition, elevation of 2-AG levels by blockade of MAGL does not cause the full spectrum of behavioral effects observed with direct (endo)cannabinoid agonists, such as hypothermia and catalepsy [94,272]. Moreover, a study by Fagundo *et al.* suggests that elevated levels of AEA are associated with an improvement of decision making and cognitive flexibility, while elevated 2-AG levels were associated with an opposite effect [274]. These studies demonstrate overt functional differences between AEA and 2-AG [275].

Although modulation of 2-AG levels does not reproduce all untoward effects observed with direct CB_1 modulation, several adverse effects have been observed upon pharmacological or genetic disruption of the major metabolic pathways of 2-AG. Chronic blockade of MAGL causes desensitization and down-regulation of CB_1 receptor, while disruption of DAGL increases anxiety, maternal neglect and reduces fear extinction. Therefore, finely-tuned rather than complete disruption of 2-AG metabolism could be a promising approach to establish a therapeutic window. The latter can be achieved via different strategies: *1)* partial blockade of major biosynthetic (DAGL) or hydrolytic (MAGL) pathways; or *2)* targeting 2-AG metabolizing enzymes that give a smaller contribution to bulk 2-AG regulation compared to DAGL and MAGL.

Lowering 2-AG levels by partial blockade of the DAGL pathway can be achieved by subtype-selective DAGL inhibitors. DAGL-β has been shown to reduce inflammatory responses *in vivo* without affecting synaptic transmission [78]. Moreover, incomplete inhibition of DAGL enzymes can be used to tweak endocannabinoid tone. Conversely, moderate increase of 2-AG could be achieved by partial inhibition of MAGL. To this aim, both MAGL and DAGL reversible inhibitors would be highly valuable tools. The *in vivo* role of enzymes that contribute in a less defined manner to metabolism (ABHD 2, 6 and 12, COX-2, LOX and CytP450), transport (FABP5) and signaling of 2-AG ($GABA_A$, adenosine A_3, TRPV1, PPARγ, and GPR55), requires further investigation. Future research on these proteins might uncover new physiological roles of 2-AG and novel approaches to modulate its (patho)physiological effects.

Acknowledgements

MM was partly supported by the Italian Ministry of Education, University and Research (MIUR) (2015KMMKBN) under competitive project PRIN 2015.

References

[1] K. Ahn, M.K. McKinney, B.F. Cravatt, Enzymatic pathways that regulate endocannabinoid signaling in the nervous system, Chem Rev 108 (5) (2008) 1687–1707.
[2] N. Murataeva, A. Straiker, K. Mackie, Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS, Br J Pharmacol 171 (6) (2014) 1379–1391.
[3] R. Mechoulam, S. Ben-Shabat, L. Hanus, M. Ligumsky, N.E. Kaminski, A.R. Schatz, et al., Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors, Biochem Pharmacol 50 (1) (1995) 83–90.
[4] T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, et al., 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain, Biochem Biophys Res Commun 215 (1) (1995) 89–97.
[5] T. Sugiura, T. Kodaka, S. Nakane, T. Miyashita, S. Kondo, Y. Suhara, et al., Evidence that the cannabinoid CB1 receptor is a 2-arachidonoylglycerol receptor. Structure-activity relationship of 2-arachidonoylglycerol, ether-linked analogues, and related compounds, J Biol Chem 274 (5) (1999) 2794–2801.
[6] J.R. Savinainen, T. Jarvinen, K. Laine, J.T. Laitinen, Despite substantial degradation, 2-arachidonoylglycerol is a potent full efficacy agonist mediating CB(1) receptor-dependent G-protein activation in rat cerebellar membranes, Br J Pharmacol 134 (3) (2001) 664–672.
[7] T. Sugiura, S. Kondo, S. Kishimoto, T. Miyashita, S. Nakane, T. Kodaka, et al., Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells, J Biol Chem 275 (1) (2000) 605–612.
[8] W. Gonsiorek, C. Lunn, X. Fan, S. Narula, D. Lundell, R.W. Hipkin, Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide, Mol Pharmacol 57 (5) (2000) 1045–1050.
[9] Y. Gaoni, R. Mechoulam, Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish, J Am Chem Soc 86 (8) (1964) 1646.
[10] M. Herkenham, A.B. Lynn, M.D. Little, M.R. Johnson, L.S. Melvin, B.R. de Costa, et al., Cannabinoid receptor localization in brain, Proc Natl Acad Sci U S A 87 (5) (1990) 1932–1936.
[11] W.A. Devane, F.A. Dysarz 3rd, M.R. Johnson, L.S. Melvin, A.C. Howlett, Determination and characterization of a cannabinoid receptor in rat brain, Mol Pharmacol 34 (5) (1988) 605–613.
[12] M.T. Viscomi, S. Oddi, L. Latini, N. Pasquariello, F. Florenzano, G. Bernardi, et al., Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway, J Neurosci 29 (14) (2009) 4564–4570.
[13] E.S. Onaivi, Commentary: Functional Neuronal CB2 Cannabinoid Receptors in the CNS, Curr Neuropharmacol 9 (1) (2011) 205–208.
[14] B.K. Atwood, A. Straiker, K. Mackie, CB(2) cannabinoid receptors inhibit synaptic

transmission when expressed in cultured autaptic neurons, Neuropharmacology 63 (4) (2012) 514–523.
[15] G.A. Cabral, L. Griffin-Thomas, Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation, Expert Rev Mol Med 11 (2009) e3.
[16] W.A. Devane, L. Hanus, A. Breuer, R.G. Pertwee, L.A. Stevenson, G. Griffin, et al., Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258 (5090) (1992) 1946–1949.
[17] R.G. Pertwee, Endocannabinoids and Their Pharmacological Actions, Handb Exp Pharmacol 231 (2015) 1–37.
[18] N. Stella, P. Schweitzer, D. Piomelli, A second endogenous cannabinoid that modulates long-term potentiation, Nature 388 (6644) (1997) 773–778.
[19] B.E. Alger, Endocannabinoids at the synapse a decade after the dies mirabilis (29 March 2001): what we still do not know, J Physiol 590 (10) (2012) 2203–2212.
[20] V. Di Marzo, D. Melck, T. Bisogno, L. De Petrocellis, Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action, Trends Neurosci 21 (12) (1998) 521–528.
[21] G.G. Muccioli, Endocannabinoid biosynthesis and inactivation, from simple to complex, Drug Discov Today 15 (11-12) (2010) 474–483.
[22] K. Mackie, Mechanisms of CB1 receptor signaling: endocannabinoid modulation of synaptic strength, Int J Obes (Lond) 30 (Suppl. 1) (2006) S19–S23.
[23] M. Rajesh, H. Pan, P. Mukhopadhyay, S. Batkai, D. Osei-Hyiaman, G. Hasko, et al., Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis, J Leukoc Biol 82 (6) (2007) 1382–1389.
[24] G. Colombo, R. Agabio, G. Diaz, C. Lobina, R. Reali, G.L. Gessa, Appetite suppression and weight loss after the cannabinoid antagonist SR 141716, Life Sci 63 (8) (1998) PL113–7.
[25] V. Di Marzo, S.K. Goparaju, L. Wang, J. Liu, S. Batkai, Z. Jarai, et al., Leptin-regulated endocannabinoids are involved in maintaining food intake, Nature 410 (6830) (2001) 822–825.
[26] M. Koch, L. Varela, J.G. Kim, J.D. Kim, F. Hernandez-Nuno, S.E. Simonds, et al., Hypothalamic POMC neurons promote cannabinoid-induced feeding, Nature 519 (7541) (2015) 45–50.
[27] W.L. Dewey, Cannabinoid pharmacology, Pharmacol Rev 38 (2) (1986) 151–178.
[28] A. Zimmer, A.M. Zimmer, A.G. Hohmann, M. Herkenham, T.I. Bonner, Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice, Proc Natl Acad Sci U S A 96 (10) (1999) 5780–5785.
[29] G. Griebel, P. Pichat, S. Beeske, T. Leroy, N. Redon, A. Jacquet, et al., Selective blockade of the hydrolysis of the endocannabinoid 2-arachidonoylglycerol impairs learning and memory performance while producing antinociceptive activity in rodents, Sci Rep 5 (2015) 7642.
[30] M. Kano, T. Ohno-Shosaku, Y. Hashimotodani, M. Uchigashima, M. Watanabe, Endocannabinoid-mediated control of synaptic transmission, Physiol Rev 89 (1) (2009) 309–380.
[31] I. Katona, T.F. Freund, Endocannabinoid signaling as a synaptic circuit breaker in neurological disease, Nat Med 14 (9) (2008) 923–930.
[32] A.J. Sanchez, A. Garcia-Merino, Neuroprotective agents: cannabinoids, Clin Immunol 142 (1) (2012) 57–67.
[33] A. Calignano, G. La Rana, A. Giuffrida, D. Piomelli, Control of pain initiation by endogenous cannabinoids, Nature 394 (6690) (1998) 277–281.
[34] M. Navarro, E. Hernandez, R.M. Munoz, I. del Arco, M.A. Villanua, M.R. Carrera, et al., Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat, Neuroreport 8 (2) (1997) 491–496.
[35] J. Haller, N. Bakos, M. Szirmay, C. Ledent, T.F. Freund, The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety, Eur J Neurosci 16 (7) (2002) 1395–1398.
[36] M.N. Hill, S. Patel, Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses, Biol Mood Anxiety Disord 3 (1) (2013) 19.
[37] R. Maldonado, O. Valverde, F. Berrendero, Involvement of the endocannabinoid system in drug addiction, Trends Neurosci 29 (4) (2006) 225–232.
[38] C. Ledent, O. Valverde, G. Cossu, F. Petitet, J.F. Aubert, F. Beslot, et al., Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice, Science 283 (5400) (1999) 401–504.
[39] D. Ogasawara, H. Deng, A. Viader, M.P. Baggelaar, A. Breman, H. den Dulk, et al., Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition, Proc Natl Acad Sci U S A 113 (1) (2016) 26–33.
[40] T. Bisogno, F. Howell, G. Williams, A. Minassi, M.G. Cascio, A. Ligresti, et al., Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain, J Cell Biol 163 (3) (2003) 463–468.
[41] L. Zhao, M.L. Yeh, E.S. Levine, Role for endogenous bdnf in endocannabinoid-mediated long-term depression at neocortical inhibitory synapses, eNeuro 2 (2) (2015).
[42] A.A. Farooqui, K.W. Rammohan, L.A. Horrocks, Isolation, characterization, and regulation of diacylglycerol lipases from the bovine brain, Ann N Y Acad Sci 559 (1989) 25–36.
[43] N. Brose, A. Betz, H. Wegmeyer, Divergent and convergent signaling by the diacylglycerol second messenger pathway in mammals, Curr Opin Neurobiol 14 (3) (2004) 328–340.
[44] T. Ohno-Shosaku, J. Shosaku, H. Tsubokawa, M. Kano, Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation, Eur J Neurosci 15 (6) (2002) 953–961.
[45] Y. Fukudome, T. Ohno-Shosaku, M. Matsui, Y. Omori, M. Fukaya, H. Tsubokawa, et al., Two distinct classes of muscarinic action on hippocampal inhibitory synapses: M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling, Eur J Neurosci 19 (10) (2004) 2682–2692.
[46] Y. Hashimotodani, T. Ohno-Shosaku, H. Tsubokawa, H. Ogata, K. Emoto, T. Maejima, et al., Phospholipase Cbeta serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal, Neuron 45 (2) (2005) 257–268.
[47] T. Maejima, S. Oka, Y. Hashimotodani, T. Ohno-Shosaku, A. Aiba, D. Wu, et al., Synaptically driven endocannabinoid release requires Ca2+-assisted metabotropic glutamate receptor subtype 1 to phospholipase Cbeta4 signaling cascade in the cerebellum, J Neurosci 25 (29) (2005) 6826–6835.
[48] T. Bisogno, F. Howell, G. Williams, A. Minassi, M.G. Cascio, A. Ligresti, et al., Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain, J Cell Biol 163 (3) (2003) 463–468.
[49] C. Aso, M. Araki, N. Ohshima, K. Tatei, T. Hirano, H. Obinata, et al., Protein purification and cloning of diacylglycerol lipase from rat brain, J Biochem 159 (6) (2016) 585–597.
[50] J.M. Inloes, K.L. Hsu, M.M. Dix, A. Viader, K. Masuda, T. Takei, et al., The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase, Proc Natl Acad Sci U S A 111 (41) (2014) 14924–14929.
[51] H. Ueda, T. Kobayashi, M. Kishimoto, T. Tsutsumi, H. Okuyama, A possible pathway of phosphoinositide metabolism through edta-insensitive phospholipase-a(1) followed by lysophosphoinositide-specific phospholipase-C in rat-brain, J Neurochem 61 (5) (1993) 1874–1881.
[52] S. Nakane, S. Oka, S. Arai, K. Waku, Y. Ishima, A. Tokumura, et al., 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain, Arch Biochem Biophys 402 (1) (2002) 51–58.
[53] Y. Gao, D.V. Vasilyev, M.B. Goncalves, F.V. Howell, C. Hobbs, M. Reisenberg, et al., Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice, J Neurosci 30 (6) (2010) 2017–2024.
[54] A. Tanimura, M. Yamazaki, Y. Hashimotodani, M. Uchigashima, S. Kawata, M. Abe, et al., The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission, Neuron 65 (3) (2010) 320–327.
[55] K.L. Hsu, K. Tsuboi, A. Adibekian, H. Pugh, K. Masuda, B.F. Cravatt, DAGLbeta inhibition perturbs a lipid network involved in macrophage inflammatory responses, Nat Chem Biol 8 (12) (2012) 999–1007.
[56] T. Bisogno, A. Mahadevan, R. Coccurello, J.W. Chang, M. Allara, Y. Chen, et al., A novel fluorophosphonate inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol with potential anti-obesity effects, Br J Pharmacol 169 (4) (2013) 784–793.
[57] T. Bisogno, J.J. Burston, R. Rai, M. Allara, B. Saha, A. Mahadevan, et al., Synthesis and pharmacological activity of a potent inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol, ChemMedChem 4 (6) (2009) 946–950.
[58] M.P. Baggelaar, P.J. Chameau, V. Kantae, J. Hummel, K.L. Hsu, F. Janssen, et al., Highly selective, reversible inhibitor identified by comparative chemoproteomics modulates diacylglycerol lipase activity in neurons, J Am Chem Soc 137 (27) (2015) 8851–8857.
[59] J.Z. Long, W.W. Li, L. Booker, J.J. Burston, S.G. Kinsey, J.E. Schlosburg, et al., Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects, Nat Chem Biol 5 (1) (2009) 37–44.
[60] M.J. Niphakis, A.B. Cognetta, J.W. Chang 3rd, M.W. Buczynski, L.H. Parsons, F. Byrne, et al., Evaluation of NHS carbamates as a potent and selective class of endocannabinoid hydrolase inhibitors, ACS Chem Neurosci 4 (9) (2013) 1322–1332.
[61] M. Brindisi, S. Maramai, S. Gemma, S. Brogi, A. Grillo, L.D. Mannelli, et al., Development and pharmacological characterization of selective blockers of 2-arachidonoyl glycerol degradation with efficacy in rodent models of multiple sclerosis and pain, J Med Chem 59 (6) (2016) 2612–2632.
[62] G. Hernandez-Torres, M. Cipriano, E. Heden, E. Bjorklund, A. Canales, D. Zian, et al., A reversible and selective inhibitor of monoacylglycerol lipase ameliorates multiple sclerosis, Angew. Chem. Int. Ed. 53 (50) (2014) 13765–13770.
[63] S. Vandevoorde, K.O. Jonsson, G. Labar, E. Persson, D.M. Lambert, C.J. Fowler, Lack of selectivity of URB602 for 2-oleoylglycerol compared to anandamide hydrolysis in vitro, Brit J Pharmacol 150 (2) (2007) 186–191.
[64] T. Bisogno, G. Ortar, S. Petrosino, E. Morera, E. Palazzo, M. Nalli, et al., Development of a potent inhibitor of 2-arachidonoylglycerol hydrolysis with antinociceptive activity in vivo, Biochim Biophys Acta 1791 (1) (2009) 53–60.
[65] W. Li, J.L. Blankman, B.F. Cravatt, A functional proteomic strategy to discover inhibitors for uncharacterized hydrolases, J Am Chem Soc 129 (31) (2007) 9594–9595.
[66] D.J. Hermanson, N.D. Hartley, J. Gamble-George, N. Brown, B.C. Shonesy, P.J. Kingsley, et al., Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation, Nat Neurosci 16 (9) (2013) 1291–1298.
[67] M. Soethoudt, U. Grether, J. Fingerle, T.W. Grim, F. Fezza, L. de Petrocellis, et al., Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity, Nat Commun 8 (2017) 13958.
[68] M. Beltramo, N. Stella, A. Calignano, S.Y. Lin, A. Makriyannis, D. Piomelli, Functional role of high-affinity anandamide transport, as revealed by selective inhibition, Science 277 (5329) (1997) 1094–1097.
[69] L. De Petrocellis, T. Bisogno, J.B. Davis, R.G. Pertwee, V. Di Marzo, Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-

like activity, FEBS Lett 483 (1) (2000) 52–56.
[70] M.L. Lopez-Rodriguez, A. Viso, S. Ortega-Gutierrez, C.J. Fowler, G. Tiger, E. de Lago, et al., Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: comparison with effects on fatty acid amidohydrolase, J Med Chem 46 (8) (2003) 1512–1522.
[71] G. Ortar, A. Ligresti, L. De Petrocellis, E. Morera, V. Di Marzo, Novel selective and metabolically stable inhibitors of anandamide cellular uptake, Biochem Pharmacol 65 (9) (2003) 1473–1481.
[72] S.M. Prescott, P.W. Majerus, Characterization of 1,2-diacylglycerol hydrolysis in human-platelets - demonstration of an arachidonoyl-monoacylglycerol intermediate, J Biol Chem 258 (2) (1983) 764–769.
[73] A.A. Farooqui, W.A. Taylor, L.A. Horrocks, Separation of bovine brain monoacylglycerol and diacylglycerol lipases by heparin sepharose affinity-chromatography, Biochem Biophys Res Commun 122 (3) (1984) 1241–1246.
[74] A.A. Farooqui, W.A. Taylor, L.A. Horrocks, Characterization and solubilization of membrane-bound diacylglycerol lipases from bovine brain, Int J Biochem 18 (11) (1986) 991–997.
[75] B.C. Shonesy, X. Wang, K.L. Rose, T.S. Ramikie, V.S. Cavener, T. Rentz, et al., CaMKII regulates diacylglycerol lipase-alpha and striatal endocannabinoid signaling, Nat Neurosci 16 (4) (2013) 456–463.
[76] M. Reisenberg, P.K. Singh, G. Williams, P. Doherty, The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signalling, Philos Trans R Soc Lond B Biol Sci 367 (1607) (2012) 3264–3275.
[77] Y. Zhou, F.V. Howell, O.O. Glebov, D. Albrecht, G. Williams, P. Doherty, Regulated endosomal trafficking of diacylglycerol lipase alpha (DAGLalpha) generates distinct cellular pools; implications for endocannabinoid signaling, Mol Cell Neurosci 76 (2016) 76–86.
[78] A. Viader, D. Ogasawara, C.M. Joslyn, M. Sanchez-Alavez, S. Mori, W. Nguyen, et al., A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation, Elife 5 (2016) e12345.
[79] T. Yoshida, M. Fukaya, M. Uchigashima, E. Miura, H. Kamiya, M. Kano, et al., Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoylglycerol, and presynaptic cannabinoid CB1 receptor, J Neurosci 26 (18) (2006) 4740–4751.
[80] I. Katona, G.M. Urban, M. Wallace, C. Ledent, K.M. Jung, D. Piomelli, et al., Molecular composition of the endocannabinoid system at glutamatergic synapses, J Neurosci 26 (21) (2006) 5628–5637.
[81] M. Uchigashima, M. Fukaya, M. Watanabe, H. Kamiya, Evidence against GABA release from glutamatergic mossy fiber terminals in the developing hippocampus, J Neurosci 27 (30) (2007) 8088–8100.
[82] M. Lafourcade, I. Elezgarai, S. Mato, Y. Bakiri, P. Grandes, O.J. Manzoni, Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex, PLoS One 2 (8) (2007) e709.
[83] R.A. Kohnz, D.K. Nomura, Chemical approaches to therapeutically target the metabolism and signaling of the endocannabinoid 2-AG and eicosanoids, Chem Soc Rev 43 (19) (2014) 6859–6869.
[84] F.J. Janssen, M. van der Stelt, Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders, Bioorg Med Chem Lett 26 (2016) 3831–3837.
[85] R.J. Bluett, R. Baldi, A. Haymer, A.D. Gaulden, N.D. Hartley, W.P. Parrish, et al., Endocannabinoid signalling modulates susceptibility to traumatic stress exposure, Nat Commun 8 (2017) 14782.
[86] H. Deng, S. Kooijman, A.M. van den Nieuwendijk, D. Ogasawara, T. van der Wel, F. van Dalen, et al., Triazole ureas act as diacylglycerol lipase inhibitors and prevent fasting-induced refeeding, J Med Chem 60 (1) (2017) 428–440.
[87] Y. Hashimotodani, T. Ohno-Shosaku, A. Tanimura, Y. Kita, Y. Sano, T. Shimizu, et al., Acute inhibition of diacylglycerol lipase blocks endocannabinoid-mediated retrograde signalling: evidence for on-demand biosynthesis of 2-arachidonoylglycerol, J Physiol 591 (Pt 19) (2013) 4765–4776.
[88] J.L. Blankman, G.M. Simon, B.F. Cravatt, A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol, Chem Biol 14 (12) (2007) 1347–1356.
[89] M. Vaughan, J.E. Berger, D. Steinberg, Hormone-sensitive lipase and monoglyceride lipase activities in adipose tissue, J Biol Chem 239 (1964) 401–409.
[90] F.P. Kupiecki, Partial purification of monoglyceride lipase from adipose tissue, J Lipid Res 7 (2) (1966) 230–235.
[91] M. Karlsson, J.A. Contreras, U. Hellman, H. Tornqvist, C. Holm, cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases, J Biol Chem 272 (43) (1997) 27218–27223.
[92] M. Karlsson, K. Reue, Y.R. Xia, A.J. Lusis, D. Langin, H. Tornqvist, et al., Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene, Gene 272 (1–2) (2001) 11–18.
[93] T.P. Dinh, T.F. Freund, D. Piomelli, A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation, Chem Phys Lipids 121 (1–2) (2002) 149–158.
[94] J.Z. Long, D.K. Nomura, B.F. Cravatt, Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism, Chem Biol 16 (7) (2009) 744–753.
[95] S. Vandevoorde, B. Saha, A. Mahadevan, R.K. Razdan, R.G. Pertwee, B.R. Martin, et al., 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 337 (1) (2005) 104–109.
[96] H. Tornqvist, L. Krabisch, P. Belfrage, Simple assay for monoacylglycerol hydrolase activity of rat adipose tissue, J Lipid Res 15 (3) (1974) 291–294.
[97] D.K. Nomura, J.Z. Long, S. Niessen, H.S. Hoover, S.W. Ng, B.F. Cravatt, Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis, Cell 140 (1) (2010) 49–61.
[98] E.Y. Dotsey, K.M. Jung, A. Basit, D. Wei, J. Daglian, F. Vacondio, et al., Peroxide-dependent mgl sulfenylation regulates 2-AG-mediated endocannabinoid signaling in brain neurons, Chem Biol 22 (5) (2015) 619–628.
[99] J.W. Chang, M.J. Niphakis, K.M. Lum, A.B. Cognetta, C. Wang 3rd, M.L. Matthews, et al., Highly selective inhibitors of monoacylglycerol lipase bearing a reactive group that is bioisosteric with endocannabinoid substrates, Chem Biol 19 (5) (2012) 579–588.
[100] P.K. Chanda, Y. Gao, L. Mark, J. Btesh, B.W. Strassle, P. Lu, et al., Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system, Mol Pharmacol 78 (6) (2010) 996–1003.
[101] J.E. Schlosburg, J.L. Blankman, J.Z. Long, D.K. Nomura, B. Pan, S.G. Kinsey, et al., Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system, Nat Neurosci 13 (9) (2010) 1113–1119.
[102] U. Taschler, F.P. Radner, C. Heier, R. Schreiber, M. Schweiger, G. Schoiswohl, et al., Monoglyceride lipase deficiency in mice impairs lipolysis and attenuates diet-induced insulin resistance, J Biol Chem 286 (20) (2011) 17467–17477.
[103] J.L. Wilkerson, M.J. Niphakis, T.W. Grim, M.A. Mustafa, R.A. Abdullah, J.L. Poklis, et al., The selective monoacylglycerol lipase inhibitor mjn110 produces opioid-sparing effects in a mouse neuropathic pain model, J Pharmacol Exp Ther 357 (1) (2016) 145–156.
[104] J.K. Makara, M. Mor, D. Fegley, S.I. Szabo, S. Kathuria, G. Astarita, et al., Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus, Nat Neurosci 8 (9) (2005) 1139–1141.
[105] A. Straiker, K. Mackie, Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones, J Physiol 569 (Pt 2) (2005) 501–517.
[106] B. Szabo, M.J. Urbanski, T. Bisogno, V. Di Marzo, A. Mendiguren, W.U. Baer, et al., Depolarization-induced retrograde synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoylglycerol, J. Physiol. Lond. 577 (1) (2006) 263–280.
[107] Y. Hashimotodani, T. Ohno-Shosaku, M. Kano, Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus, J Neurosci 27 (5) (2007) 1211–1219.
[108] B. Pan, W. Wang, J.Z. Long, D. Sun, C.J. Hillard, B.F. Cravatt, et al., 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. 331 (2) (2009) 591–597.
[109] A. Straiker, J. Wager-Miller, S.S. Hu, J.L. Blankman, B.F. Cravatt, K. Mackie, COX-2 and fatty acid amide hydrolase can regulate the time course of depolarization-induced suppression of excitation, Br J Pharmacol 164 (6) (2011) 1672–1683.
[110] B. Pan, W. Wang, P. Zhong, J.L. Blankman, B.F. Cravatt, Q.S. Liu, Alterations of endocannabinoid signaling, synaptic plasticity, learning, and memory in monoacylglycerol lipase knock-out mice, J Neurosci 31 (38) (2011) 13420–13430.
[111] A. Tanimura, M. Uchigashima, M. Yamazaki, N. Uesaka, T. Mikuni, M. Abe, et al., Synapse type-independent degradation of the endocannabinoid 2-arachidonoylglycerol after retrograde synaptic suppression, Proc Natl Acad Sci U S A 109 (30) (2012) 12195–12200.
[112] A. Busquets-Garcia, E. Puighermanal, A. Pastor, R. de la Torre, R. Maldonado, A. Ozaita, Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses, Biol Psychiatry 70 (5) (2011) 479–486.
[113] S.G. Kinsey, S.T. O'Neal, J.Z. Long, B.F. Cravatt, A.H. Lichtman, Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay, Pharmacol Biochem Behav 98 (1) (2011) 21–27.
[114] J.J. Sumislawski, T.S. Ramikie, S. Patel, Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation, Neuropsychopharmacology 36 (13) (2011) 2750–2761.
[115] S. Ghosh, L.E. Wise, Y. Chen, R. Gujjar, A. Mahadevan, B.F. Cravatt, et al., The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model, Life Sci 92 (8-9) (2013) 498–505.
[116] D.K. Nomura, B.E. Morrison, J.L. Blankman, J.Z. Long, S.G. Kinsey, M.C. Marcondes, et al., Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation, Science 334 (6057) (2011) 809–813.
[117] D.L. Ollis, E. Cheah, M. Cygler, B. Dijkstra, F. Frolow, S.M. Franken, et al., The alpha/beta hydrolase fold, Protein Eng 5 (3) (1992) 197–211.
[118] D. Navia-Paldanius, J.R. Savinainen, J.T. Laitinen, Biochemical and pharmacological characterization of human alpha/beta-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12), J Lipid Res 53 (11) (2012) 2413–2424.
[119] C.C. Lord, G. Thomas, J.M. Brown, Mammalian alpha beta hydrolase domain (ABHD) proteins: lipid metabolizing enzymes at the interface of cell signaling and energy metabolism, Biochim Biophys Acta 1831 (4) (2013) 792–802.
[120] L.E. Long, J. Lind, M. Webster, C.S. Weickert, Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex, BMC Neurosci 13 (2012) 87.
[121] K.L. Hsu, K. Tsuboi, J.W. Chang, L.R. Whitby, A.E. Speers, H. Pugh, et al., Discovery and optimization of piperidyl-1,2,3-triazole ureas as potent, selective, and in vivo-active inhibitors of alpha/beta-hydrolase domain containing 6 (ABHD6), J Med Chem 56 (21) (2013) 8270–8279.
[122] F.J. Janssen, H. Deng, M.P. Baggelaar, M. Allara, T. van der Wel, H. den Dulk, et al., Discovery of glycine sulfonamides as dual inhibitors of sn-1-diacylglycerol lipase alpha and alpha/beta-hydrolase domain 6, J Med Chem 57 (15) (2014) 6610–6622.
[123] J.Z. Patel, T.J. Nevalainen, J.R. Savinainen, Y. Adams, T. Laitinen, R.S. Runyon,

et al., Optimization of 1,2,5-thiadiazole carbamates as potent and selective ABHD6 inhibitors, ChemMedChem 10 (2) (2015) 253–265.
[124] W.R. Marrs, J.L. Blankman, E.A. Horne, A. Thomazeau, Y.H. Lin, J. Coy, et al., The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors, Nat Neurosci 13 (8) (2010) 951–957.
[125] A. Straiker, K. Mackie, Cannabinoid signaling in inhibitory autaptic hippocampal neurons, Neuroscience 163 (1) (2009) 190–201.
[126] A. Straiker, S.S. Hu, J.Z. Long, A. Arnold, J. Wager-Miller, B.F. Cravatt, et al., Monoacylglycerol lipase limits the duration of endocannabinoid-mediated depolarization-induced suppression of excitation in autaptic hippocampal neurons, Mol Pharmacol 76 (6) (2009) 1220–1227.
[127] J. Wen, R. Ribeiro, M. Tanaka, Y. Zhang, Activation of CB2 receptor is required for the therapeutic effect of ABHD6 inhibition in experimental autoimmune encephalomyelitis, Neuropharmacology 99 (2015) 196–209.
[128] A.V. Naydenov, E.A. Horne, C.S. Cheah, K. Swinney, K.L. Hsu, J.K. Cao, et al., ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 mice, Neuron 83 (2) (2014) 361–371.
[129] F. Tchantchou, Y. Zhang, Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury, J Neurotrauma 30 (7) (2013) 565–579.
[130] M. Wei, J. Zhang, M. Jia, C. Yang, Y. Pan, S. Li, et al., alpha/beta-Hydrolase domain-containing 6 (ABHD6) negatively regulates the surface delivery and synaptic function of AMPA receptors, Proc Natl Acad Sci U. S. A 113 (19) (2016) E2695–E2704.
[131] S.S. Kamat, K. Camara, W.H. Parsons, D.H. Chen, M.M. Dix, T.D. Bird, et al., Immunomodulatory lysophosphatidylserines are regulated by ABHD16A and ABHD12 interplay, Nat Chem Biol 11 (2) (2015) 164–171.
[132] D.H. Chen, A. Naydenov, J.L. Blankman, H.C. Mefford, M. Davis, Y. Sul, et al., Two novel mutations in ABHD12: expansion of the mutation spectrum in PHARC and assessment of their functional effects, Hum Mutat 34 (12) (2013) 1672–1678.
[133] T. Fiskerstrand, D. H'Mida-Ben Brahim, S. Johansson, A. M'Zahem, B.I. Haukanes, N. Drouot, et al., Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism, Am J Hum Genet 87 (3) (2010) 410–417.
[134] J.L. Blankman, J.Z. Long, S.A. Trauger, G. Siuzdak, B.F. Cravatt, ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC, Proc Natl Acad Sci U S A 110 (4) (2013) 1500–1505.
[135] X. Ding, J. Yang, S. Wang, Antisense oligonucleotides targeting abhydrolase domain containing 2 block human hepatitis B virus propagation, Oligonucleotides 21 (2) (2011) 77–84.
[136] K. Miyata, M. Nakayama, S. Mizuta, S. Hokimoto, K. Sugamura, S. Oshima, et al., Elevated mature macrophage expression of human ABHD2 gene in vulnerable plaque, Biochem Biophys Res Commun 365 (2) (2008) 207–213.
[137] S. Jin, G. Zhao, Z. Li, Y. Nishimoto, Y. Isohama, J. Shen, et al., Age-related pulmonary emphysema in mice lacking alpha/beta hydrolase domain containing 2 gene, Biochem Biophys Res Commun 380 (2) (2009) 419–424.
[138] M.R. Miller, N. Mannowetz, A.T. Iavarone, R. Safavi, E.O. Gracheva, J.F. Smith, et al., Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone, Science 352 (6285) (2016) 555–559.
[139] M. Maccarrone, M. Bari, M. Di Rienzo, A. Finazzi-Agro, A. Rossi, Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin, J Biol Chem 278 (35) (2003) 32726–32732.
[140] P. Grimaldi, M. Pucci, S. Di Siena, D. Di Giacomo, V. Pirazzi, R. Geremia, et al., The faah gene is the first direct target of estrogen in the testis: role of histone demethylase LSD1, Cell Mol Life Sci 69 (24) (2012) 4177–4190.
[141] S. Oddi, E. Dainese, F. Fezza, M. Lanuti, D. Barcaroli, V. De Laurenzi, et al., Functional characterization of putative cholesterol binding sequence (CRAC) in human type-1 cannabinoid receptor, J Neurochem 116 (5) (2011) 858–865.
[142] M. Vallee, S. Vitiello, L. Bellocchio, E. Hebert-Chatelain, S. Monlezun, E. Martin-Garcia, et al., Pregnenolone can protect the brain from cannabis intoxication, Science 343 (6166) (2014) 94–98.
[143] M. Maccarrone, E. Dainese, S. Oddi, Intracellular trafficking of anandamide: new concepts for signaling, Trends Biochem Sci 35 (11) (2010) 601–608.
[144] M. Alhouayek, J. Masquelier, P.D. Cani, D.M. Lambert, G.G. Muccioli, Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6, Proc Natl Acad Sci U. S. A 110 (43) (2013) 17558–17563.
[145] K.R. Kozak, S.W. Rowlinson, L.J. Marnett, Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2, J Biol Chem 275 (43) (2000) 33744–33749.
[146] C.A. Rouzer, L.J. Marnett, Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways, Chem Rev 111 (10) (2011) 5899–5921.
[147] J. Kim, B.E. Alger, Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus, Nat Neurosci 7 (7) (2004) 697–698.
[148] K.C. Duggan, D.J. Hermanson, J. Musee, J.J. Prusakiewicz, J.L. Scheib, B.D. Carter, et al., (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2, Nat Chem Biol 7 (11) (2011) 803–809.
[149] S.S. Hu, H.B. Bradshaw, J.S. Chen, B. Tan, J.M. Walker, Prostaglandin E2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NFkappaB activity, Br J Pharmacol 153 (7) (2008) 1538–1549.
[150] N. Sang, C. Chen, Lipid signaling and synaptic plasticity, Neuroscientist 12 (5) (2006) 425–434.
[151] N. Sang, J. Zhang, C. Chen, COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity, J Neurochem 102 (6) (2007) 1966–1977.
[152] J. Guindon, A.G. Hohmann, A physiological role for endocannabinoid-derived products of cyclooxygenase-2-mediated oxidative metabolism, Br J Pharmacol 153 (7) (2008) 1341–1343.
[153] H. Du, X. Chen, J. Zhang, C. Chen, Inhibition of COX-2 expression by endocannabinoid 2-arachidonoylglycerol is mediated via PPAR-gamma, Br J Pharmacol 163 (7) (2011) 1533–1549.
[154] J. Zhang, C. Chen, Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation, J Biol Chem 283 (33) (2008) 22601–22611.
[155] J.D. Manna, J.A. Wepy, K.L. Hsu, J.W. Chang, B.F. Cravatt, L.J. Marnett, Identification of the major prostaglandin glycerol ester hydrolase in human cancer cells, J Biol Chem 289 (49) (2014) 33741–33753.
[156] A. Andreou, I. Feussner, Lipoxygenases - Structure and reaction mechanism, Phytochemistry 70 (13–14) (2009) 1504–1510.
[157] J.S. Moody, K.R. Kozak, C. Ji, L.J. Marnett, Selective oxygenation of the endocannabinoid 2-arachidonylglycerol by leukocyte-type 12-lipoxygenase, Biochemistry 40 (4) (2001) 861–866.
[158] K.R. Kozak, R.A. Gupta, J.S. Moody, C. Ji, W.E. Boeglin, R.N. DuBois, et al., 15-Lipoxygenase metabolism of 2-arachidonylglycerol. Generation of a peroxisome proliferator-activated receptor alpha agonist, J Biol Chem 277 (26) (2002) 23278–23286.
[159] M. van der Stelt, J.A. van Kuik, M. Bari, G. van Zadelhoff, B.R. Leeflang, G.A. Veldink, et al., Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase, J Med Chem 45 (17) (2002) 3709–3720.
[160] S. Zelasko, W.R. Arnold, A. Das, Endocannabinoid metabolism by cytochrome P450 monooxygenases, Prostaglandins Other Lipid Mediat 116-117 (2015) 112–123.
[161] P. Urquhart, A. Nicolaou, D.F. Woodward, Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases, Biochim Biophys Acta 1851 (4) (2015) 366–376.
[162] J.K. Chen, J. Chen, J.D. Imig, S. Wei, D.L. Hachey, J.S. Guthi, et al., Identification of novel endogenous cytochrome p450 arachidonate metabolites with high affinity for cannabinoid receptors, J Biol Chem 283 (36) (2008) 24514–24524.
[163] D.R. McDougle, A. Kambalyal, D.D. Meling, A. Das, Endocannabinoids anandamide and 2-arachidonoylglycerol are substrates for human CYP2J2 epoxygenase, J. Pharmacol. Exp. Ther. 351 (3) (2014) 616–627.
[164] S. Nicolussi, J. Gertsch, Endocannabinoid transport revisited, Vitam Horm 98 (2015) 441–485.
[165] C.J. Fowler, Anandamide uptake explained? Trends Pharmacol Sci 33 (4) (2012) 181–185.
[166] C.J. Fowler, Transport of endocannabinoids across the plasma membrane and within the cell, FEBS J 280 (9) (2013) 1895–1904.
[167] S. Ben-Shabat, E. Fride, T. Sheskin, T. Tamiri, M.H. Rhee, Z. Vogel, et al., An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity, Eur J Pharmacol 353 (1) (1998) 23–31.
[168] V. Di Marzo, T. Bisogno, T. Sugiura, D. Melck, L. De Petrocellis, The novel endogenous cannabinoid 2-arachidonoylglycerol is inactivated by neuronal- and basophil-like cells: connections with anandamide, Biochem J 331 (Pt 1) (1998) 15–19.
[169] D. Piomelli, M. Beltramo, S. Glasnapp, S.Y. Lin, A. Goutopoulos, X.Q. Xie, et al., Structural determinants for recognition and translocation by the anandamide transporter, Proc Natl Acad Sci U S A 96 (10) (1999) 5802–5807.
[170] M. Beltramo, D. Piomelli, Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol, Neuroreport 11 (6) (2000) 1231–1235.
[171] T. Bisogno, M. MacCarrone, L. De Petrocellis, A. Jarrahian, A. Finazzi-Agro, C. Hillard, et al., The uptake by cells of 2-arachidonoylglycerol, an endogenous agonist of cannabinoid receptors, Eur J Biochem 268 (7) (2001) 1982–1989.
[172] A. Chicca, J. Marazzi, S. Nicolussi, J. Gertsch, Evidence for bidirectional endocannabinoid transport across cell membranes, J Biol Chem 287 (41) (2012) 34660–34682.
[173] M. Maccarrone, M. Bari, N. Battista, A. Finazzi-Agro, Estrogen stimulates arachidonoylethanolamide release from human endothelial cells and platelet activation, Blood 100 (12) (2002) 4040–4048.
[174] A. Chicca, S. Nicolussi, R. Bartholomaus, M. Blunder, A. Aparisi Rey, V. Petrucci, et al., Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake, Proc Natl Acad Sci U S A 114 (25) (2017) E5006–E5015.
[175] S. Nicolussi, J.M. Viveros-Paredes, M.S. Gachet, M. Rau, M.E. Flores-Soto, M. Blunder, et al., Guineensine is a novel inhibitor of endocannabinoid uptake showing cannabimimetic behavioral effects in BALB/c mice, Pharmacol Res 80 (2014) 52–65.
[176] B. Sanson, T. Wang, J. Sun, L. Wang, M. Kaczocha, I. Ojima, et al., Crystallographic study of FABP5 as an intracellular endocannabinoid transporter, Acta Crystallogr D Biol Crystallogr 70 (Pt 2) (2014) 290–298.
[177] M. Gabrielli, N. Battista, L. Riganti, I. Prada, F. Antonucci, L. Cantone, et al., Active endocannabinoids are secreted on extracellular membrane vesicles, EMBO Rep 16 (2) (2015) 213–220.
[178] E. Sigel, R. Baur, I. Racz, J. Marazzi, T.G. Smart, A. Zimmer, et al., The major central endocannabinoid directly acts at GABA(A) receptors, Proc Natl Acad Sci U S A 108 (44) (2011) 18150–18155.
[179] M. Bouaboula, S. Hilairet, J. Marchand, L. Fajas, G. Le Fur, P. Casellas,

Anandamide induced PPARgamma transcriptional activation and 3T3-L1 preadipocyte differentiation, Eur J Pharmacol 517 (3) (2005) 174–181.
[180] J.R. Lane, M.W. Beukers, T. Mulder-Krieger, A.P. Ijzerman, The endocannabinoid 2-arachidonylglycerol is a negative allosteric modulator of the human A3 adenosine receptor, Biochem Pharmacol 79 (1) (2010) 48–56.
[181] Y. Iwasaki, O. Saito, M. Tanabe, K. Inayoshi, K. Kobata, S. Uno, et al., Monoacylglycerols activate capsaicin receptor, TRPV1, Lipids 43 (6) (2008) 471–483.
[182] E. Ryberg, N. Larsson, S. Sjogren, S. Hjorth, N.O. Hermansson, J. Leonova, et al., The orphan receptor GPR55 is a novel cannabinoid receptor, Br J Pharmacol 152 (7) (2007) 1092–1101.
[183] C.E. Rockwell, N.T. Snider, J.T. Thompson, J.P. Vanden Heuvel, N.E. Kaminski, Interleukin-2 suppression by 2-arachidonyl glycerol is mediated through peroxisome proliferator-activated receptor gamma independently of cannabinoid receptors 1 and 2, Mol Pharmacol 70 (1) (2006) 101–111.
[184] S. Gessi, S. Merighi, K. Varani, E. Leung, S. Mac Lennan, P.A. Borea, The A3 adenosine receptor: an enigmatic player in cell biology, Pharmacol Ther 117 (1) (2008) 123–140.
[185] M. Kurabayashi, I. Takeyoshi, D. Yoshinari, K. Matsumoto, I. Maruyama, Y. Morishita, 2-Arachidonoylglycerol increases in ischemia-reperfusion injury of the rat liver, J Invest Surg 18 (1) (2005) 25–31.
[186] V. Carnevale, T. Rohacs, TRPV1: A target for rational drug design, Pharmaceuticals (Basel) 9 (3) (2016).
[187] D.C. McVey, P.C. Schmid, H.H. Schmid, S.R. Vigna, Endocannabinoids induce ileitis in rats via the capsaicin receptor (VR1), J Pharmacol Exp Ther 304 (2) (2003) 713–722.
[188] S.A. Golech, R.M. McCarron, Y. Chen, J. Bembry, F. Lenz, R. Mechoulam, et al., Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors, Brain Res Mol Brain Res 132 (1) (2004) 87–92.
[189] P.M. Zygmunt, A. Ermund, P. Movahed, D.A. Andersson, C. Simonsen, B.A. Jonsson, et al., Monoacylglycerols activate TRPV1—a link between phospholipase C and TRPV1, Plos One 8 (12) (2013) e81618.
[190] D.M. Shore, P.H. Reggio, The therapeutic potential of orphan GPCRs, GPR35 and GPR55, Front Pharmacol 6 (2015) 69.
[191] L. Hanus, S. Abu-Lafi, E. Fride, A. Breuer, Z. Vogel, D.E. Shalev, et al., 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor, Proc Natl Acad Sci U. S. A 98 (7) (2001) 3662–3665.
[192] T. Bisogno, D. Melck, M. Bobrov, N.M. Gretskaya, V.V. Bezuglov, L. De Petrocellis, et al., N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo, Biochem J 3 (2000) 817–824.
[193] J.D. Leggett, S. Aspley, S.R. Beckett, A.M. D'Antona, D.A. Kendall, Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors, Br J Pharmacol 141 (2) (2004) 253–262.
[194] J. Barg, E. Fride, L. Hanus, R. Levy, N. Matus-Leibovitch, E. Heldman, et al., Cannabinomimetic behavioral effects of and adenylate cyclase inhibition by two new endogenous anandamides, Eur J Pharmacol 287 (2) (1995) 145–152.
[195] I. Brown, M.G. Cascio, K.W. Wahle, R. Smoum, R. Mechoulam, R.A. Ross, et al., Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines, Carcinogenesis 31 (9) (2010) 1584–1591.
[196] A.S. Heimann, I. Gomes, C.S. Dale, R.L. Pagano, A. Gupta, L.L. de Souza, et al., Hemopressin is an inverse agonist of CB1 cannabinoid receptors, Proc Natl Acad Sci U S A 104 (51) (2007) 20588–20593.
[197] S.M. Huang, T. Bisogno, M. Trevisani, A. Al-Hayani, L. De Petrocellis, F. Fezza, et al., An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors, Proc Natl Acad Sci U S A 99 (12) (2002) 8400–8405.
[198] M. Araki, N. Ohshima, C. Aso, A. Konishi, H. Obinata, K. Tatei, et al., Enzymatic characterization of recombinant rat DDHD2: a soluble diacylglycerol lipase, J Biochem 160 (2016) 269–279.
[199] T. Tsutsumi, T. Kobayashi, H. Ueda, E. Yamauchi, S. Watanabe, H. Okuyama, Lysophosphoinositide-specific phospholipase C in rat brain synaptic plasma membranes, Neurochem Res 19 (4) (1994) 399–406.
[200] S. Zhao, Y. Mugabo, J. Iglesias, L. Xie, V. Delghingaro-Augusto, R. Lussier, et al., alpha/beta-Hydrolase domain-6-accessible monoacylglycerol controls glucose-stimulated insulin secretion, Cell Metab 19 (6) (2014) 993–1007.
[201] C. Silvestri, V. Di Marzo, The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders, Cell Metab 17 (4) (2013) 475–490.
[202] C. Ravinet Trillou, C. Delgorge, C. Menet, M. Arnone, P. Soubrie, CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity, Int J Obes Relat Metab Disord 28 (4) (2004) 640–648.
[203] Z. Pang, N.N. Wu, W. Zhao, D.C. Chain, E. Schaffer, X. Zhang, et al., The central cannabinoid CB1 receptor is required for diet-induced obesity and rimonabant's antiobesity effects in mice, Obesity (Silver Spring) 19 (10) (2011) 1923–1934.
[204] J. Simiand, M. Keane, P.E. Keane, P. Soubrie, S.R. 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset, Behav Pharmacol 9 (2) (1998) 179–181.
[205] T.C. Kirkham, C.M. Williams, F. Fezza, V. Di Marzo, Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol, Br J Pharmacol 136 (4) (2002) 550–557.
[206] D.R. Powell, J.P. Gay, N. Wilganowski, D. Doree, K.V. Savelieva, T.H. Lanthorn, et al., Diacylglycerol lipase alpha knockout mice demonstrate metabolic and behavioral phenotypes similar to those of cannabinoid receptor 1 knockout mice, Front Endocrinol (Lausanne) 6 (2015) 86.
[207] K.M. Jung, J.R. Clapper, J. Fu, G. D'Agostino, A. Guijarro, D. Thongkham, et al., 2-Arachidonoylglycerol signaling in forebrain regulates systemic energy metabolism, Cell Metab 15 (3) (2012) 299–310.
[208] G.F. Grabner, T.O. Eichmann, B. Wagner, Y. Gao, A. Farzi, U. Taschler, et al., Deletion of monoglyceride lipase in astrocytes attenuates lipopolysaccharide-induced neuroinflammation, J Biol Chem 291 (2) (2016) 913–923.
[209] S.R. Nass, J.Z. Long, J.E. Schlosburg, B.F. Cravatt, A.H. Lichtman, S.G. Kinsey, Endocannabinoid catabolic enzymes play differential roles in thermal homeostasis in response to environmental or immune challenge, J Neuroimmune Pharmacol 10 (2) (2015) 364–370.
[210] D. Panikashvili, C. Simeonidou, S. Ben-Shabat, L. Hanus, A. Breuer, R. Mechoulam, et al., An endogenous cannabinoid (2-AG) is neuroprotective after brain injury, Nature 413 (6855) (2001) 527–531.
[211] R. Mechoulam, E. Shohami, Endocannabinoids and traumatic brain injury, Mol Neurobiol 36 (1) (2007) 68–74.
[212] D. Panikashvili, N.A. Shein, R. Mechoulam, V. Trembovler, R. Kohen, A. Alexandrovich, et al., The endocannabinoid 2-AG protects the blood-brain barrier after closed head injury and inhibits mRNA expression of proinflammatory cytokines, Neurobiol Dis 22 (2) (2006) 257–264.
[213] Q. Wang, Y. Liu, J. Zhou, Neuroinflammation in Parkinson's disease and its potential as therapeutic target, Transl. Neurodeg. 4 (2015) 19.
[214] K. Hensley, Neuroinflammation in Alzheimer's disease: mechanisms, pathologic consequences, and potential for therapeutic manipulation, J. Alzheimer's Dis. 21 (1) (2010) 1–14.
[215] E. Herranz, C. Gianni, C. Louapre, C.A. Treaba, S.T. Govindarajan, R. Ouellette, et al., Neuroinflammatory component of gray matter pathology in multiple sclerosis, Ann Neurol 80 (5) (2016) 776–790.
[216] R.B. Mounsey, S. Mustafa, L. Robinson, R.A. Ross, G. Riedel, R.G. Pertwee, et al., Increasing levels of the endocannabinoid 2-AG is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, Exp Neurol 273 (2015) 36–44.
[217] J.R. Piro, D.I. Benjamin, J.M. Duerr, Y. Pi, C. Gonzales, K.M. Wood, et al., A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease, Cell Rep 1 (6) (2012) 617–623.
[218] R.Q. Chen, J. Zhang, Y. Wu, D.Q. Wang, G.P. Feng, Y.P. Tang, et al., Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease, Cell Rep 2 (5) (2012) 1329–1339.
[219] S. Valdeolivas, M.R. Pazos, T. Bisogno, F. Piscitelli, F.A. Iannotti, M. Allara, et al., The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis, rather than enhancing striatal damage, protects striatal neurons from malonate-induced death: a potential role of cyclooxygenase-2-dependent metabolism of 2-AG, Cell Death Dis 4 (2013) e862.
[220] B. Lutz, Endocannabinoid signals in the control of emotion, Curr Opin Pharmacol 9 (1) (2009) 46–52.
[221] S. Patel, C.J. Hillard, Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: Further evidence for an anxiolytic role for endogenous cannabinoid signaling, J Pharmacol Exp Ther 318 (1) (2006) 304–311.
[222] A.A. Rey, M. Purrio, M.P. Viveros, B. Lutz, Biphasic effects of cannabinoids in anxiety responses: CB1 and GABA(B) receptors in the balance of GABAergic and glutamatergic neurotransmission, Neuropsychopharmacology 37 (12) (2012) 2624–2634.
[223] C. Zanettini, L.V. Panlilio, M. Alicki, S.R. Goldberg, J. Haller, S. Yasar, Effects of endocannabinoid system modulation on cognitive and emotional behavior, Front Behav Neurosci 5 (2011) 57.
[224] J.C. Gamble-George, J.R. Conger, N.D. Hartley, P. Gupta, J.J. Sumislawski, S. Patel, Dissociable effects of CB1 receptor blockade on anxiety-like and consummatory behaviors in the novelty-induced hypophagia test in mice, Psychopharmacology (Berl) 228 (3) (2013) 401–409.
[225] M.N. Hill, B.B. Gorzalka, Enhancement of anxiety-like responsiveness to the cannabinoid CB(1) receptor agonist HU-210 following chronic stress, Eur J Pharmacol 499 (3) (2004) 291–295.
[226] F.A. Moreira, M. Grieb, B. Lutz, Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression, Best Pract Res Clin Endocrinol Metab 23 (1) (2009) 133–144.
[227] O. Valverde, M. Torrens, CB1 receptor-deficient mice as a model for depression, Neuroscience 204 (2012) 193–206.
[228] M. Morena, S. Patel, J.S. Bains, M.N. Hill, Neurobiological interactions between stress and the endocannabinoid system, Neuropsychopharmacology 41 (1) (2016) 80–102.
[229] S. Patel, C.J. Hillard, Adaptations in endocannabinoid signaling in response to repeated homotypic stress: A novel mechanism for stress habituation, Eur J Neurosci 27 (11) (2008) 2821–2829.
[230] C.J. Hillard, Stress regulates endocannabinoid-CB1 receptor signaling, Semin Immunol 26 (5) (2014) 380–388.
[231] S. Di, C.A. Itoga, M.O. Fisher, J. Solomonow, E.A. Roltsch, N.W. Gilpin, et al., Acute stress suppresses synaptic inhibition and increases anxiety via endocannabinoid release in the basolateral amygdala, J Neurosci 36 (32) (2016) 8461–8470.
[232] S. Di, R. Malcher-Lopes, V.L. Marcheselli, N.G. Bazan, J.G. Tasker, Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons, Endocrinology 146 (10) (2005) 4292–4301.
[233] N.K. Evanson, J.G. Tasker, M.N. Hill, C.J. Hillard, J.P. Herman, Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling, Endocrinology 151 (10) (2010) 4811–4819.
[234] M.N. Hill, I.N. Karatsoreos, C.J. Hillard, B.S. McEwen, Rapid elevations in limbic

endocannabinoid content by glucocorticoid hormones in vivo, Psychoneuroendocrinology 35 (9) (2010) 1333–1338.
[235] S. Kathuria, S. Gaetani, D. Fegley, F. Valino, A. Duranti, A. Tontini, et al., Modulation of anxiety through blockade of anandamide hydrolysis, Nat Med 9 (1) (2003) 76–81.
[236] S. Patel, C.T. Roelke, D.J. Rademacher, W.E. Cullinan, C.J. Hillard, Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis, Endocrinology 145 (12) (2004) 5431–5438.
[237] S. Gaetani, P. Dipasquale, A. Romano, L. Righetti, T. Cassano, D. Piomelli, et al., The endocannabinoid system as a target for novel anxiolytic and antidepressant drugs, Int Rev Neurobiol 85 (2009) 57–72.
[238] B.C. Shonesy, R.J. Bluett, T.S. Ramikie, R. Baldi, D.J. Hermanson, P.J. Kingsley, et al., Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation, Cell Rep 9 (5) (2014) 1644–1653.
[239] N.R. Sciolino, W. Zhou, A.G. Hohmann, Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats, Pharmacol Res 64 (3) (2011) 226–234.
[240] M. Aliczki, Z. Balogh, A. Tulogdi, J. Haller, The temporal dynamics of the effects of monoacylglycerol lipase blockade on locomotion, anxiety, and body temperature, Behav Pharmacol 23 (4) (2012) 348–357.
[241] M. Aliczki, D. Zelena, E. Mikics, Z.K. Varga, O. Pinter, N.V. Bakos, et al., Monoacylglycerol lipase inhibition-induced changes in plasma corticosterone levels, anxiety and locomotor activity in male CD1 mice, Horm Behav 63 (5) (2013) 752–758.
[242] I. Jenniches, S. Ternes, O. Albayram, D.M. Otte, K. Bach, L. Bindila, et al., Anxiety, stress, and fear response in mice with reduced endocannabinoid levels, Biol Psychiatry 79 (10) (2016) 858–868.
[243] T.J. De Vries, Y. Shaham, J.R. Homberg, H. Crombag, K. Schuurman, J. Dieben, et al., A cannabinoid mechanism in relapse to cocaine seeking, Nat Med 7 (10) (2001) 1151–1154.
[244] B. Le Foll, B. Forget, H.J. Aubin, S.R. Goldberg, Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies, Addict Biol 13 (2) (2008) 239–252.
[245] G.N. Balerio, E. Aso, F. Berrendero, P. Murtra, R. Maldonado, Delta9-tetrahydrocannabinol decreases somatic and motivational manifestations of nicotine withdrawal in mice, Eur J Neurosci 20 (10) (2004) 2737–2748.
[246] T. Yamaguchi, Y. Hagiwara, H. Tanaka, T. Sugiura, K. Waku, Y. Shoyama, et al., Endogenous cannabinoid, 2-arachidonoylglycerol, attenuates naloxone-precipitated withdrawal signs in morphine-dependent mice, Brain Res 909 (1-2) (2001) 121–126.
[247] D. Ramesh, G.R. Ross, J.E. Schlosburg, R.A. Owens, R.A. Abdullah, S.G. Kinsey, et al., Blockade of endocannabinoid hydrolytic enzymes attenuates precipitated opioid withdrawal symptoms in mice, J Pharmacol Exp Ther 339 (1) (2011) 173–185.
[248] P.P. Muldoon, J. Chen, J.L. Harenza, R.A. Abdullah, L.J. Sim-Selley, B.F. Cravatt, et al., Inhibition of monoacylglycerol lipase reduces nicotine withdrawal, Br J Pharmacol 172 (3) (2015) 869–882.
[249] J.R. McReynolds, E.M. Doncheck, Y. Li, O. Vranjkovic, E.N. Graf, D. Ogasawara, et al., Stress promotes drug seeking through glucocorticoid-dependent endocannabinoid mobilization in the prelimbic cortex, Biol Psychiatry (2017), http://dx.doi.org/10.1016/j.biopsych.2017.09.024.
[250] E.B. Oleson, M.V. Beckert, J.T. Morra, C.S. Lansink, R. Cachope, R.A. Abdullah, et al., Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum, Neuron 73 (2) (2012) 360–373.
[251] B. Le Foll, S.R. Goldberg, Cannabinoid CB1 receptor antagonists as promising new medications for drug dependence, J Pharmacol Exp Ther 312 (3) (2005) 875–883.
[252] M.W. Buczynski, M.A. Herman, K.L. Hsu, L.A. Natividad, C. Irimia, I.Y. Polis, et al., Diacylglycerol lipase disinhibits VTA dopamine neurons during chronic nicotine exposure, Proc Natl Acad Sci U S A 113 (4) (2016) 1086–1091.
[253] J.M. Walker, S.M. Huang, Cannabinoid analgesia, Pharmacol Ther 95 (2) (2002) 127–135.
[254] J.M. Walker, A.G. Hohmann, Cannabinoid mechanisms of pain suppression, Handb Exp Pharmacol (168) (2005) 509–554.
[255] S.G. Woodhams, D.R. Sagar, J.J. Burston, V. Chapman, The role of the endocannabinoid system in pain, Handb Exp Pharmacol 227 (2015) 119–143.
[256] N. Agarwal, P. Pacher, I. Tegeder, F. Amaya, C.E. Constantin, G.J. Brenner, et al., Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors, Nat Neurosci 10 (7) (2007) 870–879.
[257] J.D. Richardson, S. Kilo, K.M. Hargreaves, Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors, Pain 75 (1) (1998) 111–119.
[258] J. Guindon, A.G. Hohmann, The endocannabinoid system and pain, CNS Neurol Disord Drug Targets 8 (6) (2009) 403–421.
[259] J.E. Schlosburg, S.G. Kinsey, A.H. Lichtman, Targeting fatty acid amide hydrolase (FAAH) to treat pain and inflammation, AAPS J 11 (1) (2009) 39–44.
[260] P. Beaulieu, T. Bisogno, S. Punwar, W.P. Farquhar-Smith, G. Ambrosino, V. Di Marzo, et al., Role of the endogenous cannabinoid system in the formalin test of persistent pain in the rat, Eur J Pharmacol 396 (2–3) (2000) 85–92.
[261] S. Maione, L. De Petrocellis, V. de Novellis, A.S. Moriello, S. Petrosino, E. Palazzo, et al., Analgesic actions of N-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors, Br J Pharmacol 150 (6) (2007) 766–781.
[262] J. Guindon, J. Desroches, P. Beaulieu, The antinociceptive effects of intraplantar injections of 2-arachidonoyl glycerol are mediated by cannabinoid CB2 receptors, Br J Pharmacol 150 (6) (2007) 693–701.
[263] J. Desroches, J. Guindon, C. Lambert, P. Beaulieu, Modulation of the anti-nociceptive effects of 2-arachidonoyl glycerol by peripherally administered FAAH and MGL inhibitors in a neuropathic pain model, Br J Pharmacol 155 (6) (2008) 913–924.
[264] I.A. Khasabova, A. Chandiramani, C. Harding-Rose, D.A. Simone, V.S. Seybold, Increasing 2-arachidonoyl glycerol signaling in the periphery attenuates mechanical hyperalgesia in a model of bone cancer pain, Pharmacol Res 64 (1) (2011) 60–67.
[265] J. Guindon, A. Guijarro, D. Piomelli, A.G. Hohmann, Peripheral antinociceptive effects of inhibitors of monoacylglycerol lipase in a rat model of inflammatory pain, Br J Pharmacol 163 (7) (2011) 1464–1478.
[266] A.G. Hohmann, R.L. Suplita, N.M. Bolton, M.H. Neely, D. Fegley, R. Mangieri, et al., An endocannabinoid mechanism for stress-induced analgesia, Nature 435 (7045) (2005) 1108–1112.
[267] J. Guindon, Y. Lai, S.M. Takacs, H.B. Bradshaw, A.G. Hohmann, Alterations in endocannabinoid tone following chemotherapy-induced peripheral neuropathy: effects of endocannabinoid deactivation inhibitors targeting fatty-acid amide hydrolase and monoacylglycerol lipase in comparison to reference analgesics following cisplatin treatment, Pharmacol Res 67 (1) (2013) 94–109.
[268] S.G. Kinsey, D.K. Nomura, S.T. O'Neal, J.Z. Long, A. Mahadevan, B.F. Cravatt, et al., Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice, J Pharmacol Exp Ther 338 (3) (2011) 795–802.
[269] S.G. Kinsey, J.Z. Long, S.T. O'Neal, R.A. Abdullah, J.L. Poklis, D.L. Boger, et al., Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain, J Pharmacol Exp Ther 330 (3) (2009) 902–910.
[270] S.G. Kinsey, J.Z. Long, B.F. Cravatt, A.H. Lichtman, Fatty acid amide hydrolase and monoacylglycerol lipase inhibitors produce anti-allodynic effects in mice through distinct cannabinoid receptor mechanisms, J Pain 11 (12) (2010) 1420–1428.
[271] J.L. Wilkerson, S. Ghosh, D. Bagdas, B.L. Mason, M.S. Crowe, K.L. Hsu, et al., Diacylglycerol lipase beta inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain, Br J Pharmacol 173 (10) (2016) 1678–1692.
[272] J.Z. Long, D.K. Nomura, R.E. Vann, D.M. Walentiny, L. Booker, X. Jin, et al., Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo, Proc Natl Acad Sci U S A 106 (48) (2009) 20270–20275.
[273] F. Comelli, G. Giagnoni, I. Bettoni, M. Colleoni, B. Costa, 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 152 (5) (2007) 787–794.
[274] A.B. Fagundo, R. de la Torre, S. Jimenez-Murcia, Z. Aguera, A. Pastor, F.F. Casanueva, et al., Modulation of the Endocannabinoids N-Arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG) on Executive Functions in Humans, PLoS One 8 (6) (2013) e66387.
[275] V. Di Marzo, L. De Petrocellis, Why do cannabinoid receptors have more than one endogenous ligand? Philos Trans R Soc Lond B Biol Sci 367 (1607) (2012) 3216–3228.