Tsuboi et al. Inflammation and Regeneration (2018) 38:28
https://doi.org/10.1186/s41232-018-0086-5

Inflammation and Regeneration

REVIEW Open Access

Endocannabinoids and related *N*-acylethanolamines: biological activities and metabolism

Kazuhito Tsuboi[1,2*], Toru Uyama[1], Yasuo Okamoto[2] and Natsuo Ueda[1]

Abstract

The plant *Cannabis sativa* contains cannabinoids represented by Δ^9-tetrahydrocannabinol, which exert psychoactivity and immunomodulation through cannabinoid CB1 and CB2 receptors, respectively, in animal tissues. Arachidonoylethanolamide (also referred to as anandamide) and 2-arachidonoylglycerol (2-AG) are well known as two major endogenous agonists of these receptors (termed "endocannabinoids") and show various cannabimimetic bioactivities. However, only 2-AG is a full agonist for CB1 and CB2 and mediates retrograde signals at the synapse, strongly suggesting that 2-AG is physiologically more important than anandamide. The metabolic pathways of these two endocannabinoids are completely different. 2-AG is mostly produced from inositol phospholipids via diacylglycerol by phospholipase C and diacylglycerol lipase and then degraded by monoacylglycerol lipase. On the other hand, anandamide is concomitantly produced with larger amounts of other *N*-acylethanolamines via *N*-acyl-phosphatidylethanolamines (NAPEs). Although this pathway consists of calcium-dependent *N*-acyltransferase and NAPE-hydrolyzing phospholipase D, recent studies revealed the involvement of several new enzymes. Quantitatively major *N*-acylethanolamines include palmitoylethanolamide and oleoylethanolamide, which do not bind to cannabinoid receptors but exert anti-inflammatory, analgesic, and anorexic effects through receptors such as peroxisome proliferator-activated receptor α. The biosynthesis of these non-endocannabinoid *N*-acylethanolamines rather than anandamide may be the primary significance of this pathway. Here, we provide an overview of the biological activities and metabolisms of endocannabinoids (2-AG and anandamide) and non-endocannabinoid *N*-acylethanolamines.

Keywords: Lipid mediator, Endocannabinoid, 2-Arachidonoylglycerol, Anandamide, *N*-Acylethanolamine, Metabolism, Phospholipid, Phospholipase

Background

Preparations of the plant *Cannabis sativa*, such as marijuana and hashish, have been used for recreational and medical purposes for thousands of years [1]. The oldest written description of medicinal cannabis dates back to around 2350 B.C., which was found on a stone from the pyramids in Egypt. Although their psychoactivities, including euphoria, hallucination, and analgesia, have been known for a long time, the purification of Δ^9-tetrahydrocannabinol (Δ^9-THC) as the major psychoactive constituent, followed by the determination of its chemical structure, was not achieved until the 1960s [2] (Fig. 1). A large number of structurally related compounds were also isolated from cannabis and collectively referred to as cannabinoids. Synthetic analogs with more potent cannabimimetic activities were also developed and used to pharmacologically characterize a specific receptor for cannabinoids existing in rat brain crude membrane preparations [3]. The central-type CB1 cannabinoid receptor was then molecularly identified by its cDNA cloning in 1990 [4]. Subsequently, cDNA of the peripheral-type CB2 cannabinoid receptor was also found by using its sequence similarity to CB1 receptor [5]. In contrast to Δ^9-THC, cannabidiol, another major cannabinoid in cannabis, showing anti-inflammatory and anticonvulsive effects, was

* Correspondence: ktsuboi@med.kawasaki-m.ac.jp
[1]Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan
[2]Department of Pharmacology, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama 701-0192, Japan

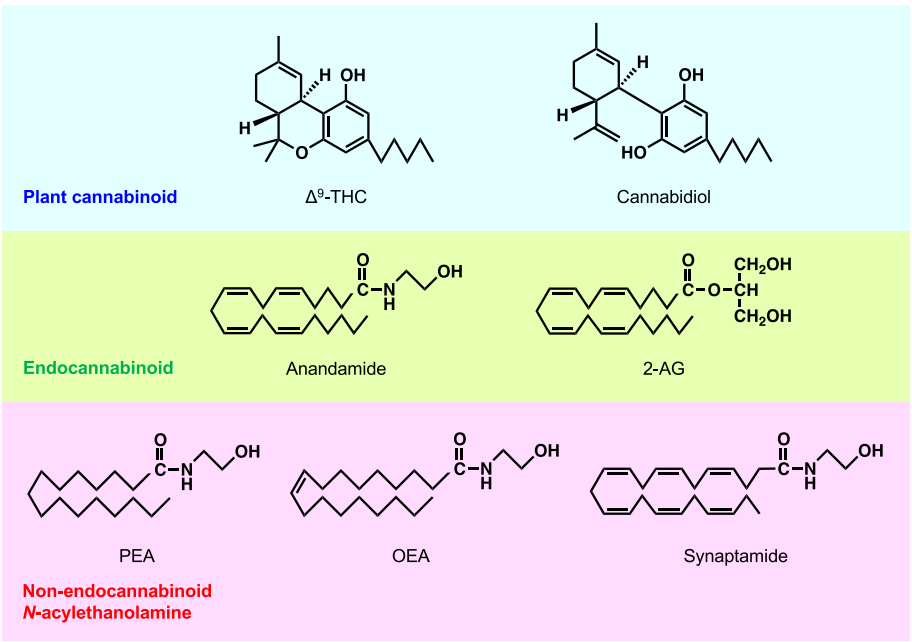

Fig. 1 Chemical structures of representative plant cannabinoids, endocannabinoids, and non-endocannabinoid *N*-acylethanolamines

almost inactive for cannabinoid receptors. Since cannabinoids are derived from the plant cannabis but not from mammals, animal tissues were expected to have endogenous counterparts capable of binding to cannabinoid receptors (later termed "endocannabinoids"). Arachidonoylethanolamide, the ethanolamide of arachidonic acid, was isolated as the first endocannabinoid from pig brain and named anandamide after "ananda," which means bliss in Sanskrit [6] (Fig. 1). Shortly after that, another derivative of arachidonic acid, 2-arachidonoylglycerol (2-AG), was also reported to show the same agonistic activity [7, 8]. It was surprising since 2-AG has been known for a long time simply as a common intermediate in the metabolisms of glycerophospholipids and triglyceride. Currently, 2-AG and anandamide are considered to be a full agonist and a partial agonist of cannabinoid receptors, respectively. Arachidonic acid is a polyunsaturated fatty acid (20:4) well known as the precursor of bioactive prostaglandins and other eicosanoids. Endocannabinoids are thus considered to be other members of arachidonic acid-related lipid mediators.

In addition to anandamide, ethanolamides of various long-chain fatty acids are also present in the body. These ethanolamides, including anandamide, are collectively referred to as *N*-acylethanolamines (Fig. 1). Ethanol-amides of saturated and monounsaturated fatty acids such as palmitic (16:0), stearic (18:0), and oleic acids (18:1) are much more abundant than anandamide in the body. These saturated and monounsaturated *N*-acylethanolamines do not bind to cannabinoid receptors, but they can activate peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor, and other receptors, leading to the exertion of biological activities including anti-inflammation and appetite suppression. In this mini-review, we will outline the biological activities and metabolisms of endocannabinoids and related *N*-acylethanolamines and emphasize that 2-AG is physiologically more important than anandamide, which appears to be a minor component concomitantly produced with cannabinoid receptor-insensitive *N*-acylethanolamines.

Biological activities of endocannabinoids

CB1 and CB2 cannabinoid receptors are G protein-coupled receptors possessing seven transmembrane helices [4, 5]. When the primary structures of the two receptors from human are compared, 44% of the amino acid residues are identical over the entire length. In their transmembrane regions, the sequence identity increases to 68%. CB1 receptor exists in abundance at the presynaptic terminals in the various regions of the brain, including substantia nigra, striatum, hippocampus, and cerebral cortex, and negatively regulates the release of the neurotransmitters. CB1 is therefore the principal receptor mediating the psychoactivities of cannabis. CB1 receptor is also present in periphery such as adrenal gland, reproductive tissues, and immune cells at lower levels. On the other hand, CB2 receptor is mainly expressed in the immune system including the spleen, thymus, and lymph nodes and is involved in the immunomodulatory effects of cannabinoids. The expression

levels of CB2 receptor in the human blood cells are in the following order: B cells > natural killer cells >> monocytes > polymorphonuclear neutrophil cells > $CD8^+$ T cells > $CD4^+$ T cells [9]. Activation of these receptors leads to a variety of cellular signal transduction such as a decrease in the cAMP level, an inhibition of N- and P/Q-type voltage-dependent Ca^{2+} channels, an opening of inwardly rectifying K^+ channels, and an activation of mitogen-activated protein kinases.

Anandamide and 2-AG exert a variety of bioactivities as cannabinoid receptor ligands, including the cannabinoid tetrad: analgesia, catalepsy, hypolocomotion, and hypothermia. They also cause bradycardia and reductions of blood and intraocular pressures. As mentioned above, anandamide is a partial agonist of CB1 receptor, while 2-AG is a full agonist of both CB1 and CB2 receptors. Furthermore, the tissue levels of 2-AG are generally hundreds to thousands of times higher than those of anandamide. Thus, 2-AG is recognized to be the true endogenous ligands of CB1 and CB2 receptors and is considered to play more important roles in vivo than anandamide [10]. However, when the anandamide-degrading enzyme, fatty acid amid hydrolase (FAAH), is pharmacologically inhibited or genetically deficient, the local concentration of anandamide would rise and could exert CB1-dependent activities. It is important that 2-AG mediates retrograde signals at the synapse [11]. 2-AG is synthesized at the postsynaptic neurons in response to the stimulus of neurotransmitters such as glutamic acid. The released 2-AG then binds to and activates presynaptic CB1 receptors and inhibits the further release of the neurotransmitter.

In addition to CB1 and CB2 receptors, pharmacological studies suggest the presence of non-CB1, non-CB2 receptors mediating the effects of cannabinoids. Although several proteins have been discussed as candidates for such potential "CB3" receptor, its existence is controversial and not yet established [12]. One of the candidates is GPR55, a G protein-coupled receptor. Δ^9-THC, a CB1/CB2 receptor agonist CP55940, anandamide, and 2-AG were reported to bind to GPR55 receptor overexpressed in human embryonic kidney HEK293s cells with nanomolar potencies, as analyzed with GTPγS binding experiments [13]. However, the pharmacological data of GPR55 gathered so far are conflicting and further analyses should be continued [14]. On the other hand, lysophosphatidylinositol, which is not a ligand of CB1 or CB2 receptor, was found to be the endogenous ligand of GPR55 [15]. Although this receptor can be activated by various molecular species of lysophosphatidylinositol having a different fatty acyl moiety at *sn*-1 or *sn*-2 position, 2-arachidonoyl-lysophosphatidylinositol is reported to be the most potent [16]. More recently, lysophosphatidylglucose was reported to be a more potent ligand of GPR55 and to mediate the correct guidance of nociceptive axons in the spinal cord [17]. Since anandamide also activates the transient receptor potential vanilloid type 1 (TRPV1) protein, a non-selective cation channel, anandamide is also regarded as one of endovanilloids [18]. However, its physiological significance as an endovanilloid is not fully elucidated.

Biological activities of non-endocannabinoid *N*-acylethanolamines

Not only anandamide but also several ethanolamides of polyunsaturated fatty acids possessing three or more double bonds, such as dihomo-γ-linolenic acid (C20:3 ω6), mead acid (C20:3 ω9), and adrenic acid (C22:4), bind to cannabinoid receptors [19, 20]. However, saturated and monounsaturated *N*-acylethanolamines do not show ligand activity for cannabinoid receptors. Instead, these non-endocannabinoid *N*-acylethanolamines exert biological activities through different receptors. Importantly, non-endocannabinoid *N*-acylethanolamines such as palmitoylethanolamide (PEA, C16:0 *N*-acylethanolamine), stearoylethanolamide (C18:0 *N*-acylethanolamine), oleoyl-ethanolamide (OEA, C18:1 *N*-acylethanolamine), and linoleoylethanolamide (C18:2 *N*-acylethanolamine) are much more abundant than anandamide in most animal tissues. Biosynthetic enzymes for *N*-acylethanolamines so far reported do not show selectivity for anandamide over other *N*-acylethanolamine species. Thus, anandamide could be concomitantly produced as a kind of by-product of non-endocannabinoid *N*-acylethanolamines.

PEA is a food component known for more than 60 years [21]. This molecule was isolated from soybean lecithin, egg yolk, and peanut meal and was shown to exert an anti-inflammatory activity in a local passive joint anaphylaxis assay in the guinea pig [22, 23]. Since then, PEA has been shown to have anti-inflammatory, analgesic, anti-epileptic, and neuroprotective actions [24, 25]. These actions are mediated at least in part by PPARα. Preclinical and clinical studies suggest that PEA is potentially useful in a wide range of therapeutic areas, including eczema, pain, and neurodegeneration [26]. In the USA and Europe, PEA is currently marketed as a nutraceutical, a food supplement, or a food for medical purposes, depending on the country, which is effective for chronic pain represented by neuropathic pain. PEA is also a constituent of cream marketed for dry, irritated, and reactive skin. Although it was reported that PEA could activate GPR55 [13], this agonist activity has not been fully elucidated.

OEA is known to have an anorexic activity in experimental animals [27]. Administration of OEA produces satiety and reduces body weight gain [28]. OEA binds with high affinity to PPARα, and these effects are not observed with PPARα-deficient mice, suggesting that the

anorexic action of OEA is mediated by PPARα. Since OEA is proposed to be produced from the digested dietary fat in the enterocytes of small intestine [29], endogenous OEA may mediate the satiety after the intake of fatty food. Furthermore, the dysfunction of OEA signaling could contribute to overweight and obesity. Thus, analogs of OEA and the inhibitors of OEA-degrading enzymes, such as FAAH, could be expected as novel anti-obesity drugs. OEA is also reported to activate GPR119 in vitro [30]. This G protein-coupled receptor was expressed in the intestinal L-cells, which secrete glucagon-like peptide-1 (GLP-1), and intraileal administration of OEA to rats was found to increase plasma GLP-1 levels [31]. However, the anorexic action of OEA was observed even in GPR119-deficient mice [32], suggesting that GPR119 system is not essential for OEA-induced satiety. Although OEA was reported to be a weak agonist of TRPV1 [33], TRPV1-deficient mice also exhibit OEA-induced suppression of appetite [34]. On the other hand, TRPV1 is suggested to mediate the reducing effects of OEA on levodopa (L-DOPA)-induced dyskinesia [35]. Thus, the OEA-TRPV1 system might be an effective target for the treatment of L-DOPA-induced dyskinesias.

Docosahexaenoylethanolamide (C22:6 *N*-acylethanol-amine) is the ethanolamide of docosahexaenoic acid, one of major ω3 polyunsaturated fatty acids, and is referred to as synaptamide. At nanomolar concentrations, synaptamide promotes neurogenesis, neurite outgrowth, and synaptogenesis in developing neurons [36]. Recently, these actions were shown to be mediated by the activation of GPR110, which is also termed as adhesion G protein-coupled receptor F1 (ADGRF1) [37]. Although the physiological significance in the development of neurons and cognitive functions remains elusive, the synaptamide-GPR110 system could be a novel target for the treatment of neurodevelopmental diseases. Furthermore, the beneficial effects of docosahexaenoic acid on the central nervous system might be partly mediated by the generation of synaptamide.

Metabolism of endocannabinoid 2-arachidonoylglycerol

Although 2-AG is biosynthesized in multiple pathways, all the pathways start from *sn*-2 arachidonic acid-containing glycerophospholipids, which are abundant in cell membranes and therefore suitable as starting materials [10] (Fig. 2). The main precursors are inositol phospholipids with 2-arachidonoyl group such as 2-arachidonoyl-phosphatidylinositol 4,5-bisphosphate. The inositol phospholipids are hydrolyzed by phospholipase C to form 2-arachidonoyl-diacylglycerol, which is further deacylated by *sn*-1-specific diacylglycerol lipase (DAGL) to yield 2-AG (Fig. 2). Glycerophospholipids other than inositol phospholipids, such as phosphatidic acid and phosphatidylcholine (PC), could also be hydrolyzed to 2-arachidonoyl-diacylglycerol [38–40]. Human DAGL has two isozymes, DAGLα and DAGLβ. Their cDNAs were cloned in 2003 [41]. In DAGLα-deficient mice, the retrograde suppression of synaptic transmission is lost with concomitant decreases in 2-AG levels of brain and spinal cord [42–44]. Thus, DAGLα is suggested to be the main biosynthetic enzyme of 2-AG in the central nervous system. While the role of DAGL in the hydrolysis of membrane phospholipid-derived *sn*-1,2-diacylglycerol species is well established, it was described that DAGL enzymes are unlikely to be involved in the degradation of *rac*-1,3- or *sn*-2,3-diacylglycerol that originates from lipolysis-driven triacylglycerol breakdown [45].

Alternatively, 2-arachidonoyl-phosphatidylinositol could be hydrolyzed at *sn*-1 position by an intracellular phospholipase A_1, DDHD domain containing 1, previously known as phosphatidic acid-preferring phospholipase A_1 [46] (Fig. 2). The formed 2-arachidonoyl-lysophos phatidylinositol is known as an endogenous agonist of GPR55 as described above and is further hydrolyzed to 2-AG by a phospholipase C-type enzyme. Furthermore, 2-AG could be produced by dephosphorylation of arachidonic acid-containing lysophosphatidic acid (LPA) [47]. These alternative pathways, which bypass 2-arachidonoyl-diacylglycerol and therefore do not involve DAGL, seemed to play a certain role in vivo since ~15% of 2-AG levels remained even in the cerebral cortex of DAGLα/β double-knockout mice, compared to those of wild-type mice [44].

The major degradative pathway of 2-AG is considered to be the hydrolysis to arachidonic acid and glycerol (Fig. 2). This reaction can be catalyzed by multiple enzymes, including monoacylglycerol lipase (MAGL), FAAH, α/β-hydrolase domain containing (ABHD) 6, and ABHD12. The relative contribution of these enzymes differs among tissues and cells. In mouse brain, MAGL is responsible for around 85% of the 2-AG-hydrolyzing activity in vitro [48]. cDNA of this enzyme was cloned from mouse adipocytes in 1997 [49]. MAGL hydrolyzes not only 2-AG but also other 2-monoacylglycerols and 1-monoacylglycerols. Pharmacological inhibition of MAGL in mice caused CB1-dependent symptoms including analgesia, hypothermia, and hypomotility, indicating the central role of this enzyme in the degradation of 2-AG in the brain [50]. Although MAGL-deficient mice exhibited increased 2-AG levels in the brain and spinal cord, no abnormalities in nociception, body temperature, or spontaneous locomotion were observed in MAGL-deficient mice [51, 52]. This apparent discrepancy is supposed to be due to the desensitization of CB1 receptor. Apart from the endocannabinoid system,

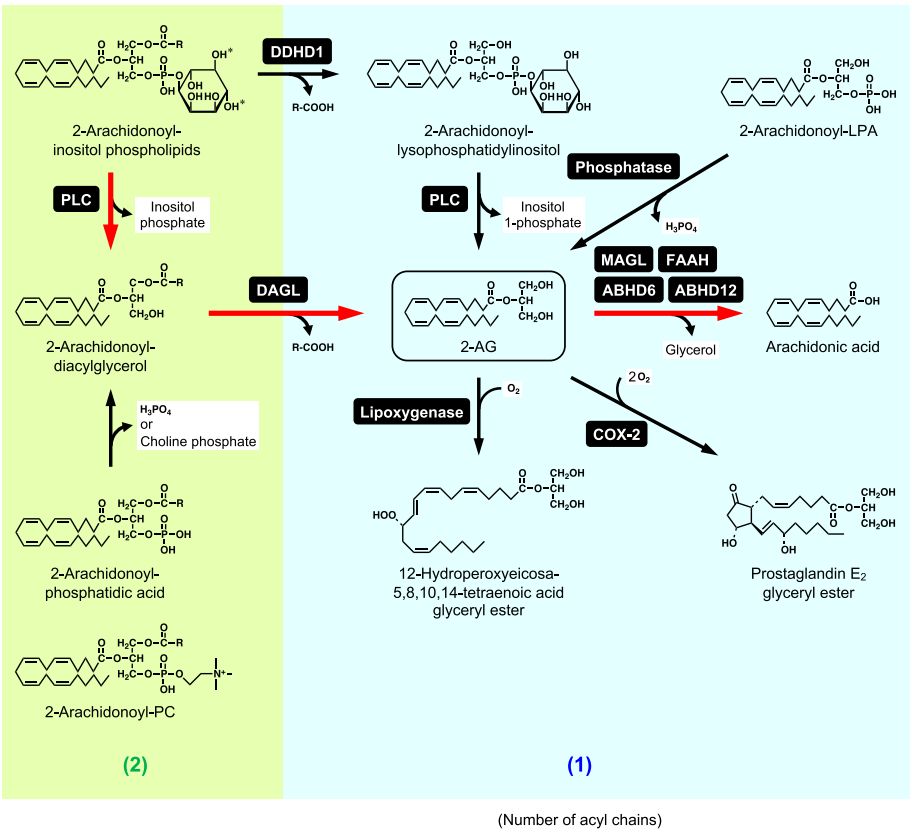

Fig. 2 Metabolism of 2-AG. Red thick arrows represent the major pathway. H_2O is omitted in the hydrolytic reactions. Two hydroxyl groups indicated by asterisks are phosphorylated in the case of 2-arachidonoyl-phosphatidylinositol 4,5-bisphosphate. Numbers of acyl chains per molecule are indicated in parentheses. *COX-2* cyclooxygenase-2, *DDHD1* DDHD domain containing 1, *PLC* phospholipase C

MAGL-dependent generation of arachidonic acid from 2-AG is also responsible for the production of prostaglandins that promote neuroinflammation and fever generation in the brain [53, 54].

FAAH plays the central role in the degradation of anandamide, another endocannabinoid, as described in the following section. FAAH also hydrolyzes 2-AG. However, the role of FAAH in 2-AG degradation in vivo is considered to be minor. In mouse microglia BV-2 cells, ABHD6 controls the accumulation of 2-AG, and knockdown of ABHD6 increases the efficacy with which 2-AG can stimulate CB2-mediated cell migration [55]. ABHD6 is also expressed postsynaptically in neurons, and the specific inhibitor of ABHD6 as well as MAGL inhibitors induces CB1-dependent long-term depression. As another metabolic route of 2-AG, the arachidonoyl moiety of 2-AG could be directly oxygenated by cyclooxygenase-2 and lipoxygenases to produce glycerol esters of prostaglandins and hydroperoxyeicosatetraenoic acids, respectively (Fig. 2). Glycerol esters of prostaglandins are reported to show biological activities including anti-inflammatory, pro-inflammatory, and hyperalgesic effects [56].

The pathway consisting of phospholipase C, DAGL, and MAGL has attracted attention due to the formation of two second messengers, diacylglycerol and inositol trisphosphate, and the release of free arachidonic acid from phospholipid, which may be utilized to generate eicosanoids. The major pathway for the biosynthesis and degradation of 2-AG completely agrees with this pathway, and this fact implies its multifunctionality of this pathway.

Metabolism of *N*-acylethanolamines

In animal tissues, a series of *N*-acylethanolamines including anandamide is biosynthesized through common metabolic pathways starting from glycerophospholipids (Fig. 3). The pathways are largely different from the aforementioned 2-AG metabolism. First, *sn*-1 acyl group of glycerophospholipids such as PC is transferred to the amino group of ethanolamine glycerophospholipids represented by phosphatidylethanolamine (PE). This *N*-acylation of PE results in the generation of *N*-acyl-PE (NAPE), which is a unique type of glycerophospholipid in that three fatty acyl chains exist per molecule. The responsible enzyme *N*-acyltransferase has been known

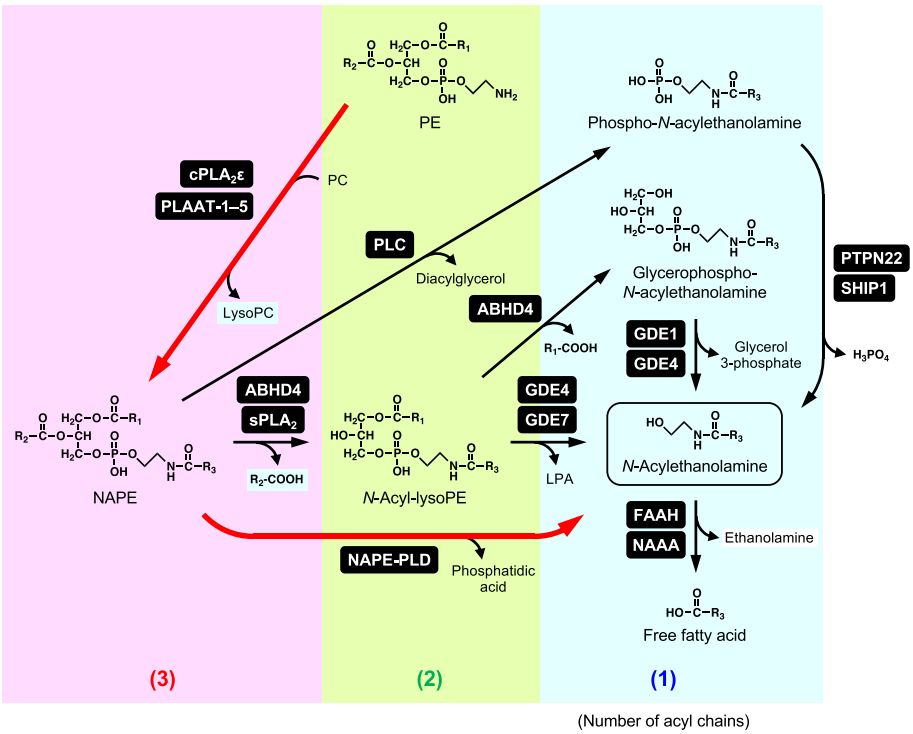

Fig. 3 Metabolism of *N*-acylethanolamines. Red thick arrows represent the canonical pathway. H_2O is omitted in the hydrolytic reactions. Numbers of acyl chains per molecule are indicated in parentheses. *cPLA*$_2$ cytosolic phospholipase A_2, *PLC* phospholipase C, *sPLA*$_2$ secretory phospholipase A_2

to be stimulated by Ca^{2+} since the 1980s [57–59] and called as Ca-dependent *N*-acyltransferase (Ca-NAT) to distinguish from Ca-independent enzymes discussed later. However, its molecular characterization was achieved only recently when mouse Ca-NAT was identified by an activity-based proteomic approach as isoform ε of cytosolic phospholipase A_2 (PLA2G4E) [60]. Our group then found that human ortholog has two isoforms, which are distinguished by the length and amino acid residues of their N-terminal sequences, and that both isoforms show Ca-NAT activity [61]. We also revealed that this Ca^{2+}-dependent activity is further enhanced by phosphatidylserine. In agreement with the fact that the *sn*-1 position of glycerophospholipids is mostly occupied by a saturated or monounsaturated fatty acid, the anandamide precursor *N*-arachidonoyl-PE is a minor component among various NAPEs with different *N*-acyl species. This may be the main reason why anandamide is a minor component of *N*-acylethanolamines.

Apart from Ca-NAT, we found that all of the five members of HRAS-like suppressor (HRASLS) family, HRASLS1–5, have Ca^{2+}-independent *N*-acyltransferase activities as well as phospholipase A_1/A_2 activities [62–67]. These family members were previously reported as tumor suppressor genes, negatively regulating the oncogene *Ras*. On the basis of their enzyme activities, we proposed to rename them phospholipase A/acyltransferase (PLAAT)-1–5, respectively [66]. Among the members, PLAAT-1, PLAAT-2, and PLAAT-5 have relatively high *N*-acyltransferase activities over phospholipase A_1/A_2 activities [67, 68], suggesting their roles in the Ca^{2+}-independent generation of NAPE in vivo.

The formed NAPE is then hydrolyzed to release *N*-acylethanolamines by a phospholipase D (PLD)-type enzyme, NAPE-PLD (Fig. 3). Our group purified this enzyme from rat heart and cloned its cDNAs from human, mouse, and rat [69]. The enzyme specifically hydrolyzes NAPE, but not PE or PC. The primary structure of NAPE-PLD shows that this enzyme belongs to the metallo-β-lactamase family and has no sequence similarity with other PLDs, which typically hydrolyze PC to phosphatidic acid and choline. Thus, NAPE-PLD is distinct from other PLDs in both structure and catalytic function.

In addition to the one-step *N*-acylethanolamine-forming reaction catalyzed by NAPE-PLD, the presence of multi-step pathways via *N*-acyl-lysoPE was suggested using dog brain preparations in the 1980s [58] (Fig. 3). The cDNA cloning of NAPE-PLD enabled the generation of NAPE-PLD$^{-/-}$ mice, and three groups including ours independently established the mutant mice and confirmed

the presence of the multi-step NAPE-PLD-independent pathways in brain and other mammalian tissues [70–73]. In these pathways, one *O*-acyl chain is first eliminated from NAPE, resulting in the formation of *N*-acyl-lysoPE. This reaction occurred in vitro by group IB, IIA, and V of secretory phospholipase A_2s [74]. *N*-Acyl-lysoPE can be further *O*-deacylated to glycerophospho-*N*-acylethanol-amine. ABHD4 was found to function as a hydrolase catalyzing these sequential *O*-deacylation reactions from NAPE to glycerophospho-*N*-acylethanolamine via *N*-acyl-lysoPE [75]. Glycerophospho-*N*-acylethanolamine is further hydrolyzed to form *N*-acylethanolamine by two members of the glycerophosphodiesterase (GDE) family, GDE1 [76] and GDE4 [77, 78]. Alternatively, *N*-acyl-lysoPE can be directly converted to *N*-acyletha-nolamine by lysophospholipase D-type enzymes. In this reaction, LPA is also formed as another product. This lysophospholipase D-type reaction seems particularly important when the substrate *N*-acyl-lysoPE is "plasmalogen-type" containing a lipase-resistant alkenyl chain at *sn*-1 position of the glycerol backbone [71]. We found that GDE4 and GDE7 have this lysophospholipase D-type activity [77, 78]. Interestingly, the divalent cation requirement for the activity differs among GDE members: GDE1 and GDE4 are Mg^{2+}-dependent while GDE7 is Ca^{2+}-dependent. In addition, an anandamide-forming pathway through phosphoanandamide (anandamide phosphate) was previously suggested in the brain and macrophages. This pathway is composed of phospholipase C and phosphatase. Tyrosine phosphatase PTPN22 and inositol 5′-phosphatase SHIP1 were shown to have this phosphatase activity while the phospholipase C has not yet been identified [79, 80]. The reverse reaction of FAAH can synthesize anandamide from free arachidonic acid and ethanolamine in vitro [81, 82]. The analysis of FAAH-deficient mice suggests the in vivo production of anandamide through this route [83].

N-Acylethanolamines are degraded by the hydrolysis to free fatty acids and ethanolamine (Fig. 3). FAAH catalyzes this reaction, and this enzyme has been extensively studied since its cDNA cloning in 1996 [84]. FAAH is a membrane-bound serine hydrolase, belonging to the amidase signature family. The catalytic activity is higher at neutral and alkaline pH. FAAH hydrolyzes various *N*-acylethanolamines with a higher reactivity toward anandamide. FAAH is ubiquitously present in various tissues with abundant expressions in the brain and liver, and FAAH-deficient mice exhibit increased tissue levels of various *N*-acylethanolamines including anandamide, suggesting the central role of this enzyme in the degradation of *N*-acylethanolamines [85, 86]. Specific FAAH inhibitors have been developed, and they are expected as novel therapeutic drugs against a variety of symptoms such as pain, depression, and anxiety. These beneficial effects are mostly considered to result from the increased tissue levels of anandamide acting as an endocannabinoid. However, FAAH also hydrolyzes cannabinoid receptor-insensitive *N*-acylethanolamines and other bioactive fatty acid amides such as oleamide and *N*-acyltaurine. Thus, we should be careful in interpreting the molecular mechanisms of the phenotype caused by genetic and pharmacological depletion of FAAH. The dual inhibitors of FAAH and MAGL have also been developed, and they increase both anandamide and 2-AG levels to mimic the pharmacological activities of CB1 receptor agonist in vivo [87, 88]. FAAH-2, an isozyme having around 20% of amino acid sequence identity with FAAH (FAAH-1), is also present in primates, but not in rodents [89], and this enzyme localizes on lipid droplets in cells [90].

N-Acylethanolamine-hydrolyzing acid amidase (NAAA) is a lysosomal enzyme hydrolyzing *N*-acylethanolamines only at acidic pH [91]. We cloned cDNA of this enzyme from rat lung in 2005 [92]. NAAA belongs to the cholylglycine hydrolase family and shows no sequence similarity with FAAH. Acid ceramidase is another lysosomal enzyme belonging to this family, which hydrolyzes ceramide under acidic conditions. NAAA and acid ceramidase have significant amino acid sequence similarity (33–34% identity), and their catalytic activities partially overlap each other: NAAA hydrolyzes ceramide at a low rate while acid ceramidase also has an *N*-acylethanolamine-hydrolyzing activity. NAAA is present in various tissues with abundant expression in macrophages and prostate [93, 94]. In contrast to the preference of FAAH to anandamide, the best substrate of NAAA in vitro is PEA. In consistence with the anti-inflammatory action of PEA, the administration of specific NAAA inhibitors suppresses inflammatory responses in rodent models with increased local PEA levels [95–99]. NAAA-deficient mice also show a strongly reduced inflammatory reaction, compared to wild-type animals [99]. Thus, NAAA inhibitors may have the therapeutic potential as novel anti-inflammatory drugs.

Conclusions

In this mini-review, we outlined the biological activities and metabolisms of two representative endocannabinoids, 2-AG and anandamide, as well as cannabinoid receptor-insensitive *N*-acylethanolamines. Pharmacological and biochemical analyses now reveal that 2-AG is a more important endocannabinoid than anandamide. The classical pathway composed of phospholipase C, DAGL, and MAGL attracts much attention again as the central pathway for the metabolism of 2-AG functioning as the major endocannabinoid. On the other hand, anandamide is produced in a small amount along with PEA and OEA, which are cannabinoid receptor-insensitive,

but quantitatively major bioactive *N*-acylethanolamines. The presence of Ca-NAT and NAPE-PLD, which appear to be exclusively responsible for the biosynthesis of *N*-acylethanolamines, strongly suggest the physiological importance of *N*-acylethanolamines and their precursors *N*-acyl-PEs. Thus, further studies on biological activities of various *N*-acylethanolamines are eagerly required, which include the development of specific enzyme inhibitors and analyses of gene-disrupted animals for the enzymes involved. As the research in this field progresses, the metabolic pathways have been found to be more complex than previously considered. Recently found enzymes, such as PLAAT and GDE family members, have not been fully elucidated and their roles in vivo must be clarified.

Abbreviations

2-AG: 2-Arachidonoylglycerol; ABHD: α/β-Hydrolase domain containing; Ca-NAT: Ca-dependent *N*-acyltransferase; DAGL: Diacylglycerol lipase; FAAH: Fatty acid amide hydrolase; GDE: Glycerophosphodiesterase; GLP-1: Glucagon-like peptide-1; HRASLS: HRAS-like suppressor; LPA: Lysophosphatidic acid; MAGL: Monoacylglycerol lipase; NAAA: *N*-Acylethanolamine-hydrolyzing acid amidase; NAPE: *N*-Acyl-phosphatidylethanolamine; OEA: Oleoylethanolamide; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PEA: Palmitoylethanolamide; PLAAT: Phospholipase A/acyltransferase; PLD: Phospholipase D; PPARα: Peroxisome proliferator-activated receptor α; TRPV1: Transient receptor potential vanilloid type 1

Funding

KT was supported by the JSPS KAKENHI Grant Number JP17K01852 and Ryobi Teien Memory Foundation.

Authors' contributions

KT wrote the manuscript, and TU, YO, and NU improved it. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Received: 30 July 2018 Accepted: 5 September 2018
Published online: 01 October 2018

References

1. Ligresti A, De Petrocellis L, Di Marzo V. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. Physiol Rev. 2016;96:1593–659.
2. Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc. 1964;86:1646–7.
3. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988;34:605–13.
4. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990;346:561–4.
5. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;365:61–5.
6. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258:1946–9.
7. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol. 1995;50:83–90.
8. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun. 1995;215:89–97.
9. Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem. 1995;232:54–61.
10. Sugiura T, Kishimoto S, Oka S, Gokoh M. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog Lipid Res. 2006;45:405–46.
11. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009;89:309–80.
12. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacol Rev. 2010;62:588–631.
13. Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007;152:1092–101.
14. Sharir H, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther. 2010;126:301–13.
15. Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. Biochem Biophys Res Commun. 2007;362:928–34.
16. Oka S, Toshida T, Maruyama K, Nakajima K, Yamashita A, Sugiura T. 2-Arachidonoyl-*sn*-glycero-3-phosphoinositol: a possible natural ligand for GPR55. J Biochem. 2009;145:13–20.
17. Guy AT, Nagatsuka Y, Ooashi N, Inoue M, Nakata A, Greimel P, et al. Glycerophospholipid regulation of modality-specific sensory axon guidance in the spinal cord. Science. 2015;349:974–7.
18. van der Stelt M, Di Marzo V. Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. Eur J Biochem. 2004;271:1827–34.
19. Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. Proc Natl Acad Sci U S A. 1993;90:7656–60.
20. Priller J, Briley EM, Mansouri J, Devane WA, Mackie K, Felder CC. Mead ethanolamide, a novel eicosanoid, is an agonist for the central (CB1) and peripheral (CB2) cannabinoid receptors. Mol Pharmacol. 1995;48:288–92.
21. Keppel Hesselink JM, de Boer T, Witkamp RF. Palmitoylethanolamide: a natural body-own anti-inflammatory agent, effective and safe against influenza and common cold. Int J Inflam. 2013;2013:151028.
22. Kuehl FA Jr, Jacob TA, Ganley OH, Ormond RE, Meisinger MAP. The identification of N-(2-hydroxyethyl)-palmitamide as a naturally occurring anti-inflammatory agent. J Am Chem Soc. 1957;79:5577–8.
23. Ganley OH, Graessle OE, Robinson HJ. Anti-inflammatory activity on compounds obtained from egg yolk, peanut oil, and soybean lecithin. J Lab Clin Med. 1958;51:709–14.
24. Mattace Raso G, Russo R, Calignano A, Meli R. Palmitoylethanolamide in CNS health and disease. Pharmacol Res. 2014;86:32–41.
25. Petrosino S, Di Marzo V. The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. Br J Pharmacol. 2017;174:1349–65.
26. Gabrielsson L, Mattsson S, Fowler CJ. Palmitoylethanolamide for the treatment of pain: pharmacokinetics, safety and efficacy. Br J Clin Pharmacol. 2016;82:932–42.
27. Pavón FJ, Serrano A, Romero-Cuevas M, Alonso M, Rodríguez de Fonseca F. Oleoylethanolamide: a new player in peripheral control of energy metabolism. Therapeutic implications. Drug Discov Today Dis Mech. 2010;7:e175–83.

28. Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodríguez de Fonseca F, et al. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-α. Nature. 2003;425:90–3.
29. Piomelli D. A fatty gut feeling. Trends Endocrinol Metab. 2013;24:332–41.
30. Overton HA, Babbs AJ, Doel SM, Fyfe MCT, Gardner LS, Griffin G, et al. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metab. 2006;3:167–75.
31. Lauffer LM, Iakoubov R, Brubaker PL. GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. Diabetes. 2009;58:1058–66.
32. Lan H, Vassileva G, Corona A, Liu L, Baker H, Golovko A, et al. GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis. J Endocrinol. 2009;201:219–30.
33. Wang X, Miyares RL, Ahern GP. Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J Physiol. 2005;564:541–7.
34. Lo Verme J, Gaetani S, Fu J, Oveisi F, Burton K, Piomelli D. Regulation of food intake by oleoylethanolamide. Cell Mol Life Sci. 2005;62:708–16.
35. González-Aparicio R, Moratalla R. Oleoylethanolamide reduces L-DOPA-induced dyskinesia via TRPV1 receptor in a mouse model of Parkinson's disease. Neurobiol Dis. 2014;62:416–25.
36. Kim H-Y, Spector AA. *N*-Docosahexaenoylethanolamine: a neurotrophic and neuroprotective metabolite of docosahexaenoic acid. Mol Aspects Med. 2018. https://doi.org/10.1016/j.mam.2018.03.004.
37. Lee J-W, Huang BX, Kwon H, Rashid MA, Kharebava G, Desai A, et al. Orphan GPR110 (ADGRF1) targeted by *N*-docosahexaenoylethanolamine in development of neurons and cognitive function. Nat Commun. 2016;7:13123.
38. Bisogno T, Melck D, De Petrocellis L, Di Marzo V. Phosphatidic acid as the biosynthetic precursor of the endocannabinoid 2-arachidonoylglycerol in intact mouse neuroblastoma cells stimulated with ionomycin. J Neurochem. 1999;72:2113–9.
39. Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K, et al. Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB_2 receptor-dependent mechanism. Mol Pharmacol. 2004;65:999–1007.
40. Oka S, Yanagimoto S, Ikeda S, Gokoh M, Kishimoto S, Waku K, et al. Evidence for the involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in 12-*O*-tetradecanoylphorbol-13-acetate-induced acute inflammation in mouse ear. J Biol Chem. 2005;280:18488–97.
41. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, 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. 2003;163:463–8.
42. Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci. 2010;30:2017–24.
43. Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, et al. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase a mediates retrograde suppression of synaptic transmission. Neuron. 2010;65:320–7.
44. Yoshino H, Miyamae T, Hansen G, Zambrowicz B, Flynn M, Pedicord D, et al. Postsynaptic diacylglycerol lipase mediates retrograde endocannabinoid suppression of inhibition in mouse prefrontal cortex. J Physiol. 2011;589:4857–84.
45. Eichmann TO, Lass A. DAG tales: the multiple faces of diacylglycerol–stereochemistry, metabolism, and signaling. Cell Mol Life Sci. 2015;72:3931–52.
46. Yamashita A, Kumazawa T, Koga H, Suzuki N, Oka S, Sugiura T. Generation of lysophosphatidylinositol by DDHD domain containing 1 (DDHD1): possible involvement of phospholipase D/phosphatidic acid in the activation of DDHD1. Biochim Biophys Acta. 1801;2010:711–20.
47. Nakane S, Oka S, Arai S, Waku K, Ishima Y, Tokumura A, 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. 2002;402:51–8.
48. Blankman JL, Simon GM, Cravatt BF. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. Chem Biol. 2007;14:1347–56.
49. Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. J Biol Chem. 1997;272:27218–23.
50. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, et al. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol. 2009;5:37–44.
51. Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, Kinsey SG, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nat Neurosci. 2010;13:1113–9.
52. Chanda PK, Gao Y, Mark L, Btesh J, Strassle BW, Lu P, et al. Monoacylglycerol lipase activity is a critical modulator of the tone and integrity of the endocannabinoid system. Mol Pharmacol. 2010;78:996–1003.
53. Nomura DK, Morrison BE, Blankman JL, Long JZ, Kinsey SG, Marcondes MCG, et al. Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. Science. 2011;334:809–13.
54. Kita Y, Yoshida K, Tokuoka SM, Hamano F, Yamazaki M, Sakimura K, et al. Fever is mediated by conversion of endocannabinoid 2-arachidonoylglycerol to prostaglandin E_2. PLOS ONE. 2015;e0133663:10.
55. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci. 2010;13:951–7.
56. Alhouayek M, Muccioli GG. COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. Trends Pharmacol Sci. 2014;35:284–92.
57. Natarajan V, Reddy PV, Schmid PC, Schmid HHO. *N*-acylation of ethanolamine phospholipids in canine myocardium. Biochim Biophys Acta. 1982;712:342–55.
58. Natarajan V, Schmid PC, Reddy PV, Zuzarte-Augustin ML, Schmid HHO. Biosynthesis of *N*-acylethanolamine phospholipids by dog brain preparations. J Neurochem. 1983;41:1303–12.
59. Schmid HHO, Schmid PC, Natarajan V. *N*-Acylated glycerophospholipids and their derivatives. Prog Lipid Res. 1990;29:1–43.
60. Ogura Y, Parsons WH, Kamat SS, Cravatt BF. A calcium-dependent acyltransferase that produces *N*-acyl phosphatidylethanolamines. Nat Chem Biol. 2016;12:669–71.
61. Hussain Z, Uyama T, Kawai K, Binte Mustafiz SS, Tsuboi K, Araki N, et al. Phosphatidylserine-stimulated production of *N*-acyl-phosphatidylethanolamines by Ca^{2+}-dependent *N*-acyltransferase. Biochim Biophys Acta. 1863;2018:493–502.
62. Jin X-H, Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Discovery and characterization of a Ca^{2+}-independent phosphatidylethanolamine *N*-acyltransferase generating the anandamide precursor and its congeners. J Biol Chem. 2007;282:3614–23.
63. Jin X-H, Uyama T, Wang J, Okamoto Y, Tonai T, Ueda N. cDNA cloning and characterization of human and mouse Ca^{2+}-independent phosphatidylethanolamine *N*-acyltransferases. Biochim Biophys Acta. 1791;2009:32–8.
64. Uyama T, Morishita J, Jin X-H, Okamoto Y, Tsuboi K, Ueda N. The tumor suppressor gene H-Rev107 functions as a novel Ca^{2+}-independent cytosolic phospholipase $A_{1/2}$ of the thiol hydrolase type. J Lipid Res. 2009;50:685–93.
65. Uyama T, Jin X-H, Tsuboi K, Tonai T, Ueda N. Characterization of the human tumor suppressors TIG3 and HRASLS2 as phospholipid-metabolizing enzymes. Biochim Biophys Acta. 1791;2009:1114–24.
66. Shinohara N, Uyama T, Jin X-H, Tsuboi K, Tonai T, Houchi H, et al. Enzymological analysis of the tumor suppressor A-C1 reveals a novel group of phospholipid-metabolizing enzymes. J Lipid Res. 2011;52:1927–35.
67. Uyama T, Ikematsu N, Inoue M, Shinohara N, Jin X-H, Tsuboi K, et al. Generation of *N*-acylphosphatidylethanolamine by members of the phospholipase A/acyltransferase (PLA/AT) family. J Biol Chem. 2012;287:31905–19.
68. Uyama T, Inoue M, Okamoto Y, Shinohara N, Tai T, Tsuboi K, et al. Involvement of phospholipase A/acyltransferase-1 in *N*-acylphosphatidylethanolamine generation. Biochim Biophys Acta. 2013;1831:1690–701.
69. Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. J Biol Chem. 2004;279:5298–305.

70. Leung D, Saghatelian A, Simon GM, Cravatt BF. Inactivation of *N*-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. Biochemistry. 2006;45:4720–6.
71. Tsuboi K, Okamoto Y, Ikematsu N, Inoue M, Shimizu Y, Uyama T, et al. Enzymatic formation of *N*-acylethanolamines from *N*-acylethanolamine plasmalogen through *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. Biochim Biophys Acta. 2011;1811:565–77.
72. Leishman E, Mackie K, Luquet S, Bradshaw HB. Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. Biochim Biophys Acta. 1861;2016:491–500.
73. Inoue M, Tsuboi K, Okamoto Y, Hidaka M, Uyama T, Tsutsumi T, et al. Peripheral tissue levels and molecular species compositions of *N*-acyl-phosphatidylethanolamine and its metabolites in mice lacking *N*-acyl-phosphatidylethanolamine-specific phospholipase D. J Biochem. 2017;162:449–58.
74. Sun Y-X, Tsuboi K, Okamoto Y, Tonai T, Murakami M, Kudo I, et al. Biosynthesis of anandamide and *N*-palmitoylethanolamine by sequential actions of phospholipase A_2 and lysophospholipase D. Biochem J. 2004;380:749–56.
75. Simon GM, Cravatt BF. Endocannabinoid biosynthesis proceeding through glycerophospho-*N*-acyl ethanolamine and a role for α/β-hydrolase 4 in this pathway. J Biol Chem. 2006;281:26465–72.
76. Simon GM, Cravatt BF. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-*N*-acyl ethanolamine precursors in mouse brain. J Biol Chem. 2008;283:9341–9.
77. Tsuboi K, Okamoto Y, Rahman IAS, Uyama T, Inoue T, Tokumura A, et al. Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: a possible involvement in bioactive *N*-acylethanolamine biosynthesis. Biochim Biophys Acta. 2015;1851:537–48.
78. Rahman IAS, Tsuboi K, Hussain Z, Yamashita R, Okamoto Y, Uyama T, et al. Calcium-dependent generation of *N*-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. Biochim Biophys Acta. 2016;1861:1881–92.
79. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, et al. A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A. 2006;103:13345–50.
80. Liu J, Wang L, Harvey-White J, Huang BX, Kim H-Y, Luquet S, et al. Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology. 2008;54:1–7.
81. Arreaza G, Devane WA, Omeir RL, Sajnani G, Kunz J, Cravatt BF, et al. The cloned rat hydrolytic enzyme responsible for the breakdown of anandamide also catalyzes its formation via the condensation of arachidonic acid and ethanolamine. Neurosci Lett. 1997;234:59–62.
82. Katayama K, Ueda N, Katoh I, Yamamoto S. Equilibrium in the hydrolysis and synthesis of cannabimimetic anandamide demonstrated by a purified enzyme. Biochim Biophys Acta. 1999;1440:205–14.
83. Patel S, Carrier EJ, Ho WSV, Rademacher DJ, Cunningham S, Reddy DS, et al. The postmortal accumulation of brain *N*-arachidonylethanolamine (anandamide) is dependent upon fatty acid amide hydrolase activity. J Lipid Res. 2005;46:342–9.
84. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996;384:83–7.
85. Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, et al. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci U S A. 2001;98:9371–6.
86. Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH. Functional disassociation of the central and peripheral fatty acid amide signaling systems. Proc Natl Acad Sci U S A. 2004;101:10821–6.
87. Nomura DK, Blankman JL, Simon GM, Fujioka K, Issa RS, Ward AM, et al. Activation of the endocannabinoid system by organophosphorus nerve agents. Nat Chem Biol. 2008;4:373–8.
88. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, et al. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proc Natl Acad Sci U S A. 2009;106:20270–5.
89. Wei BQ, Mikkelsen TS, McKinney MK, Lander ES, Cravatt BF. A second fatty acid amide hydrolase with variable distribution among placental mammals. J Biol Chem. 2006;281:36569–78.
90. Kaczocha M, Glaser ST, Chae J, Brown DA, Deutsch DG. Lipid droplets are novel sites of *N*-acylethanolamine inactivation by fatty acid amide hydrolase-2. J Biol Chem. 2010;285:2796–806.
91. Tsuboi K, Takezaki N, Ueda N. The *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). Chem Biodivers. 2007;4:1914–25.
92. Tsuboi K, Sun Y-X, Okamoto Y, Araki N, Tonai T, Ueda N. Molecular characterization of *N*-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. J Biol Chem. 2005;280:11082–92.
93. Tsuboi K, Zhao L-Y, Okamoto Y, Araki N, Ueno M, Sakamoto H, et al. Predominant expression of lysosomal *N*-acylethanolamine-hydrolyzing acid amidase in macrophages revealed by immunochemical studies. Biochim Biophys Acta. 2007;1771:623–32.
94. Wang J, Zhao L-Y, Uyama T, Tsuboi K, Wu X-X, Kakehi Y, et al. Expression and secretion of *N*-acylethanolamine-hydrolysing acid amidase in human prostate cancer cells. J Biochem. 2008;144:685–90.
95. Solorzano C, Zhu C, Battista N, Astarita G, Lodola A, Rivara S, et al. Selective *N*-acylethanolamine-hydrolyzing acid amidase inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. Proc Natl Acad Sci U S A. 2009;106:20966–71.
96. Ribeiro A, Pontis S, Mengatto L, Armirotti A, Chiurchiù V, Capurro V, et al. A potent systemically active *N*-acylethanolamine acid amidase inhibitor that suppresses inflammation and human macrophage activation. ACS Chem Biol. 2015;10:1838–46.
97. Petrosino S, Ahmad A, Marcolongo G, Esposito E, Allarà M, Verde R, et al. Diacerein is a potent and selective inhibitor of palmitoylethanolamide inactivation with analgesic activity in a rat model of acute inflammatory pain. Pharmacol Res. 2015;91:9–14.
98. Bonezzi FT, Sasso O, Pontis S, Realini N, Romeo E, Ponzano S, et al. An important role for *N*-acylethanolamine acid amidase in the complete Freund's adjuvant rat model of arthritis. J Pharmacol Exp Ther. 2016;356:656–63.
99. Sasso O, Summa M, Armirotti A, Pontis S, De Mei C, Piomelli D. The N-acylethanolamine acid amidase inhibitor ARN077 suppresses inflammation and pruritus in a mouse model of allergic dermatitis. J Invest Dermatol. 2018;138:562–9.