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Roles of Endocannabinoids and Endocannabinoid-Like Molecules in Energy Homeostasis and Metabolic Regulation: A Nutritional Perspective

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N-acylethanolamine, anandamide, appetite, 2-arachidonoylglycerol, oleoylethanolamide, peroxisome proliferator-activated receptor

Abstract

The endocannabinoid system is involved in signal transduction in mammals. It comprises principally G protein-coupled cannabinoid receptors and their endogenous agonists, called endocannabinoids, as well as the enzymes and transporters responsible for the metabolism of endocannabinoids. Two arachidonic acid–containing lipid molecules, arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol, function as endocannabinoids. *N*-acylethanolamines and monoacylglycerols, in which the arachidonic acid chain is replaced with a saturated or monounsaturated fatty acid, are not directly involved in the endocannabinoid system but exhibit agonistic activities for other receptors. These endocannabinoid-like molecules

include palmitoylethanolamide, oleoylethanolamide (OEA), and 2-oleoylglycerol. Endocannabinoids stimulate feeding behavior and the anabolism of lipids and glucose, while OEA suppresses appetite. Both central and peripheral systems are included in these nutritional and metabolic contexts. Therefore, they have potential in the treatment and prevention of obesity. We outline the structure, metabolism, and biological activities of endocannabinoids and related molecules, and focus on their involvement in energy homeostasis and metabolic regulation.

Contents

1. INTRODUCTION

Arachidonoylethanolamide (AEA): an endocannabinoid acting as a partial agonist of CB1; also called anandamide

The complete endocannabinoid system is a complex one that is responsible for lipid signaling in humans and other mammals. It mainly comprises four physiological modules: ligands, receptors, enzymes, and transporters. Two arachidonic acid–containing lipid molecules, arachidonoylethanolamide (AEA) and 2-arachidonoylglycerol (2-AG), are representative endogenous ligands called endocannabinoids, and the G protein-coupled cannabinoid receptors CB1 (central-type) and CB2 (peripheral-type) are principal receptors (**Figure 1**) (127, 139). The pharmacological effects of endocannabinoids are close to those of Δ^9-tetrahydrocannabinol, the major psychoactive ingredient of cannabis, because both molecules function as cannabinoid

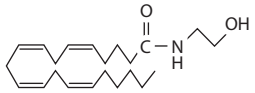
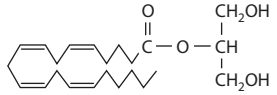
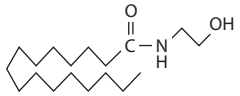
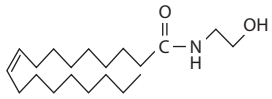
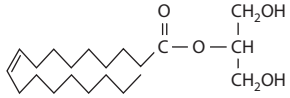
	Principal receptors	*N*-Acylethanolamines	Monoacylglycerols
Endocannabinoids	CB1, CB2, etc.	**AEA (Anandamide)** 	**2-AG** 
Endocannabinoid-like molecules	PPAR-α, GPR55, GPR119, TRPV1, etc.	**PEA**  **OEA** 	**2-OG** 

Figure 1

Endocannabinoids and endocannabinoid-like molecules. Endocannabinoids and endocannabinoid-like molecules are structurally similar to each other but bind to different receptors.

receptor agonists. In recent years, endocannabinoids have attracted the attention of biochemical and medicinal researchers because of their various activities, such as analgesic, relaxation-inducing, and hunger-stimulatory effects (1). Endocannabinoids differ from other neurotransmitters and hormones in that they are enzymatically produced on demand from membrane phospholipids in various tissues, including the nervous system and immune cells (83). Therefore, endocannabinoids can be defined as a class of lipid mediators, similar to prostanoids, leukotrienes, and lysophospholipids.

Structurally, AEA and 2-AG are members of the *N*-acylethanolamine (NAE) and monoacylglycerol (MAG) groups, respectively (139). Members containing polyunsaturated fatty acids with three or more double bonds, such as AEA and 2-AG, act as endocannabinoids, while those with a saturated or monounsaturated fatty acid, including palmitoylethanolamide (PEA), oleoylethanolamide (OEA), and 2-oleoylglycerol (2-OG), are not directly involved in the endocannabinoid system, but they exhibit some agonistic activities for noncannabinoid receptors, such as peroxisome proliferator-activated receptors (PPARs), the G protein-coupled receptors GPR55 and GPR119, and transient receptor potential vanilloid-1 (TRPV1) (**Figure 1**). The latter molecules, hereafter referred to as endocannabinoid-like molecules, have also attracted attention in the development of painkillers and antiobesity drugs (69).

Endocannabinoids and endocannabinoid-like molecules exhibit numerous biological activities (**Table 1**). However, their physiological significance remains unclear. Based on evidence in biochemistry and pharmacology, it is now reasonable to conclude that 2-AG is a physiologically true endocannabinoid (137), while AEA is a by-product of quantitatively major NAEs, such as PEA and OEA (138). However, the physiological roles of these major NAEs have not been elucidated in as much detail as the role of 2-AG as an endocannabinoid. The endocannabinoid system stimulates feeding behavior and the anabolism of lipids and glucose (144). In contrast, OEA suppresses appetite, mainly through PPAR-α (140). Therefore, endocannabinoids and OEA both have potential in the treatment and prevention of obesity. We herein outline the structure, metabolism, and biological activities of both MAGs and NAEs, with special reference to their involvement in energy homeostasis and metabolic regulation.

2-Arachidonoylglycerol (2-AG): an endocannabinoid acting as a full agonist of both CB1 and CB2

CB1: the central type of cannabinoid receptor, a G protein-coupled receptor binding to phytocannabinoids and endocannabinoids, mainly expressed in the central nervous system

CB2: the peripheral type of cannabinoid receptor, a G protein-coupled receptor expressed in immune cells and other peripheral tissues

***N*-Acylethanolamine (NAE):** a fatty acid amide in which a fatty acyl chain is covalently bound to ethanolamine through an amide bond

Table 1 Physiological targets and biological functions of the endocannabinoids and endocannabinoid-like molecules

	Molecule	Target receptors	Biological functions
Endocannabinoids	AEA	Partial agonist of CB1 and weak agonist of CB2	Brain: enhances food intake and facilitates energy storage (146) Liver: activates lipogenic enzyme and accumulates fat (104) Pancreas: stimulates insulin secretion (116) Gastrointestinal tract: decreases satiation, gastric acid secretion and motility (144) Adipose tissue: stimulates LPL activity and decreases adiponectin level (123) Muscle: reduces insulin-mediated glucose uptake and oxygen consumption. Decreases AMPK activity and fatty acid oxidation (32, 123)
	2-AG	Full agonist of CB1 and CB2	
Endocannabinoid-like molecules	OEA	PPAR-α, PPAR-γ, GPR119, TRPV1, CD36	Regulates food intake and body weight in mammals (140) Extends life span in *Caenorhabditis elegans* (37) Provides cytoprotection in neurons and other peripheral tissues such as heart and liver (143) Aids in management of pain and inflammation (143)
	PEA	PPAR-α, PPAR-γ, GPR119, TRPV1, GPR55	Acts as an anti-inflammatory and analgesic compound (66) Shows neuroprotective and antiepileptic properties (66) Enhances the activity of AEA through "entourage effect" (58) Maintains mild and neuropathic pain (96)
	LEA	PPAR-α, PPAR-γ, GPR119, TRPV1	Inhibits the electrically induced twitch response of mouse vas deferens (109) Causes catalepsy in mice without any effect on sleeping time (109)
	SEA	SEA-binding site, not coupled to G proteins	Regulates immunological and inflammatory responses (16, 82) Functions as an antioxidative and membrane-protective compound (16, 82) Stimulates AP-1 transcriptional activity by MAPK/ERK pathway in mice (35)
	DHEA	GPR110 and partial agonist of CB1 and CB2	Stimulates neurite growth and synaptogenesis in mouse hippocampal neurons (67) Develops neurons and cognitive function (106) Shows anti-inflammatory effects (106)
	2-OG	GPR119	Produces GLP-1 by ingestion of *sn*-2 oleic acid-enriched fats (57)
	2-PG	GPR119	Enhances the ability of 2-AG to bind with CB1 or CB2 and to inhibit adenylyl cyclase (6) Modulates pain sensitivity by interacting with other endocannabinoids (143)
	2-LG	GPR119	Potentiates the action of endocannabinoids by inhibiting the degradation of 2-AG (6)

Abbreviations: 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; 2-PG, 2-palmitoylglycerol; AEA, arachidonoylethanolamide; AMPK, AMP-activated protein kinase; DHEA, docosahexaenoylethanolamide; LEA, linoleoylethanolamide; LPL, lipoprotein lipase; MAPK/ERK, mitogen-activated protein kinase/extracellular signal–regulated kinase; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; SEA, stearoylethanolamide; TRPV1, transient receptor potential vanilloid-1.

2. STRUCTURES AND BIOLOGICAL ACTIVITIES OF ENDOCANNABINOIDS AND ENDOCANNABINOID-LIKE MOLECULES

2.1. Structures and Activities of 2-AG and Other 2-MAGs

Monoacylglycerol (MAG): produced in large quantities from dietary and stored fat; also called monoglyceride

MAGs, also called monoglycerides, are esters of the trihydric alcohol glycerol, in which one of the hydroxyl groups is esterified with a long-chain fatty acid. MAGs may be broadly divided into two groups depending on the position of the ester bond. Among three stereochemical forms, 1- and 3-isomers cannot be distinguished from each other and are termed 1(3)-MAG or α-MAG, while the 2-isomer 2-MAG, also called β-MAG, is distinct from 1(3)-MAG. MAGs are

nonionic molecules with both hydrophilic and hydrophobic properties, which are important in the food, cosmetic, pharmaceutical, and chemical industries (27). In 1995, 2-AG, a MAG with an arachidonic acid chain (20:4) at the *sn*-2 position of the glycerol backbone, was isolated from the dog gut (93) and rat brain (128) as an endocannabinoid. In contrast to AEA, 2-AG functions as an effective and full agonist for both CB1 and CB2 (**Table 1**), which makes it a true endogenous ligand for both receptors (127). In the brain, the tissue where 2-AG is the most abundant, it functions as a synaptic retrograde signaling molecule (64). This molecule has been detected in other tissues, such as the heart, liver, spleen, lung, kidney, plasma, colon, and small intestine. Moreover, 2-AG has been identified in numerous biofluids, including human milk and blood serum from normal donors and patients with endotoxin shock (31). Various tissues also contain 1(3)-AG, which binds weakly with cannabinoid receptors (127).

2-Palmitoylglycerol (2-PG), 2-OG, and 2-linoleoylglycerol (2-LG) are representative 2-MAGs, containing palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2), respectively. These MAGs are not considered to be endocannabinoids because they have no or low affinities for cannabinoid receptors. However, some biological activities, including the modulation of the activities of endocannabinoids, have been reported (6, 130). Large amounts of MAG are produced as end products in the intestinal breakdown of dietary fat by pancreatic lipase and other lipases. MAGs are then absorbed by intestinal cells and transformed again into triacylglycerols (TAGs) via the MAG pathway before being moved through lymph to the liver (36). MAGs are also formed in adipose tissues during the mobilization of stored TAG and in the peripheral vascular system from TAG by lipoprotein lipase (15).

In humans, 2-OG is found primarily in the intestinal lumen. A 2011 study reported that a dietary intake of approximately 80–100 g of fat each day resulted in the accumulation of approximately 15–20 g of 2-OG in the intestinal lumen during digestion. Therefore, 2-OG is more abundantly produced from the diet than OEA (57). Although 2-OG was also detected in brain homogenates, the amount was approximately one-tenth that of 2-AG (114). Similar to OEA, 2-OG may function as an agonist of GPR119, the biological significance of which is discussed below.

2-PG does not bind directly to cannabinoid receptors or inhibit adenylyl cyclase. However, 2-PG was shown to enhance the ability of 2-AG to bind with CB1 or CB2 and inhibit adenylyl cyclase (6). This "entourage effect" was attributed to the blockage of the hydrolysis and reabsorption pathways that normally operate to rapidly reduce endocannabinoid levels after discharge (6). The inhibitory effects of 2-AG on tumor necrosis factor-α were also enhanced by the coexistence of 2-PG in rodent macrophages (44). Furthermore, 2-PG and related endogenous fatty acid derivatives, such as oleamide, PEA, and a group of arachidonoyl amino acids, may be vital in the modulation of pain sensitivity by interacting with endocannabinoids (91, 143).

Ben-Shabat et al. reported that 2-LG potentiated the effects of endocannabinoids by inhibiting the degradation of 2-AG through functional antagonism in both neuronal and hematopoietic cell lines (6). However, using cultured murine hippocampal neurons, Lu et al. demonstrated that 2-LG did not potentiate the effects of 2-AG or AEA in a CB1-dependent manner, whereas it exerted antagonistic effects (81). They also assessed the effects of 2-LG on human CB1 receptor activity with a very sensitive and computable cell-based reporter assay using β-lactamase enzyme. The findings obtained indicated that 2-LG functions as a partial agonist of CB1 and moderately inhibits the activity of both 2-AG and AEA.

2.2. Structures and Activities of AEA and Other NAEs

NAEs are fatty acid amides in which one of numerous types of fatty acyl groups is connected to the nitrogen atom of ethanolamine. NAEs are biologically defined as a group of lipid signaling molecules that are involved in the mammalian endocannabinoid system as well as in various

Palmitoylethanolamide (PEA): a palmitic acid–containing NAE that exerts anti-inflammatory and analgesic effects as a ligand of PPAR-α

Oleoylethanolamide (OEA): an oleic acid–containing NAE that exerts appetite-suppressing effects as a ligand of PPAR-α

2-Oleoylglycerol (2-OG): abundantly produced during oleic acid–containing fat digestion; exhibits ligand activity for GPR119

GPR119: a G protein-coupled receptor expressed in gut L cells, which secretes GLP-1 in response to OEA and 2-OG

Peroxisome proliferator-activated receptor-α (PPAR-α): a receptor involved in lipid metabolism, leading to a decrease in blood triacylglycerol levels

physiological processes, including nutrient sensing and energy homeostasis (30). NAEs were suggested to have therapeutic potential in the treatment of microbial infections due to their cell-protective and stress-combating effects and anti-inflammatory properties (69). They were also reported to be useful in the development of liposomal formulations for drug delivery and the specific targeting of molecules (63).

Fatty acid amide hydrolase (FAAH): a membrane-bound enzyme hydrolyzing various fatty acid amides, including bioactive NAEs such as AEA, OEA, and PEA

AEA was discovered in 1992 as the first endocannabinoid and was named anandamide, which is derived from the Sanskrit word for ecstasy and excitement (26). It was initially detected in the brain and then in many other tissues (26). AEA shares numerous properties with the phytocannabinoid Δ^9-tetrahydrocannabinol, but it acts as a partial agonist of CB1 and as a weak agonist or sometimes an antagonist of CB2 (110). Because of these findings, along with the findings that AEA is a minor component among NAEs in most tissues and that its endogenous levels are generally markedly lower than 2-AG levels, AEA is considered a physiologically less important endocannabinoid than 2-AG.

OEA does not exhibit cannabimimetic activity but functions as a lipid mediator that controls various biological functions (**Table 1**). Its regulation of food intake and body weight is well known in mammals (140). OEA functions as an agonist of PPAR-α, is profusely generated in the small intestine, and inhibits food intake via the activation of PPAR-α. The roles of OEA in energy homeostasis and metabolic regulation are discussed below. OEA was also reported to extend the life span of a nematode (*Caenorhabditis elegans*) with the collaboration of lysosomal signaling molecules (37). Additional biological roles for OEA have been reported, including cytoprotection in neurons and other peripheral tissues as well as the management of pain and inflammation (143). Cytoprotective roles against heart and liver toxicities have also been demonstrated (108).

PEA was isolated as an anti-inflammatory and analgesic compound from a phospholipid fraction of egg yolk and peanut meal and was subsequently reported to possess neuroprotective and antiepileptic properties (66). PEA is present in the majority of mammalian tissues as a major NAE. PEA was initially thought to function as an agonist of CB2; however, this was ultimately rejected (6). Although PEA lacks affinity for CB1 and CB2, cellular PEA and OEA were proposed to enhance the activity of AEA through the "entourage effect" (58). Alternatively, PEA was reported to be an agonist of PPAR-α, GPR55, and GPR119 (50, 69). The anti-inflammatory and analgesic effects of PEA are currently considered to be mostly mediated by PPAR-α. PEA is commercially available in a capsule with other ingredients for the treatment of temporary mild pain. PEA is also combined with some antioxidant molecules for the better treatment of neuropathic pain and enhancement of locomotor function (96).

Linoleoylethanolamide (LEA) was initially detected in the porcine brain, rodent peritoneal J774 macrophages, and N18 neuroblastoma cells as a fatty acid amide hydrolase (FAAH) inhibitor (120). Pertwee and coworkers reported that LEA inhibited the electrically induced twitch response of the mouse vas deferens by interacting with cannabinoid receptors (109). However, LEA only weakly interacted with CB1 and CB2 and was approximately fourfold weaker than AEA at inducing catalepsy in mice without any effects on sleeping times.

Stearoylethanolamide (SEA) appears to be ubiquitous in all mammals with its three polymorphic structures (148). SEA possesses some medicinal properties that regulate immunological and inflammatory responses as well as antioxidative and membrane-protective functionalities (16, 82). A study demonstrated that SEA did not bind to CB1 or CB2; instead, it bound to a new site that was most abundant in the cortex and not coupled to G proteins (84). SEA was also found to stimulate AP-1 transcriptional activity by the MAPK/ERK (mitogen-activated protein kinase/extracellular signal–regulated kinase) pathway in mouse epidermal cells (35). In addition, in C6 glioma cells, 1 μM SEA induced apoptosis in a time-dependent manner (85).

Docosahexaenoylethanolamide (DHEA) is an ethanolamide of docosahexaenoic acid (22:6) and is also designated as synaptamide. DHEA was initially discovered in the rat brain and bovine retina. A recent study suggested that a fish oil–rich diet increased DHEA levels in the rat jejunum but decreased levels of AEA, OEA, and PEA (94). DHEA acted as a relatively weak ligand for both CB1 and CB2 (18) and inhibited the hydrolysis of AEA, which suggested that DHEA is recognized by FAAH but serves as a poor substrate (94). In mouse hippocampal neurons with enhanced glutamatergic synaptic activity, DHEA stimulated neurite growth and synaptogenesis (**Table 1**), which were completely independent of the interaction with cannabinoid receptors (67). DHEA was recently shown to bind to GPR110 in order to exert various anti-inflammatory effects and stimulate the development of neurons and cognitive function (106).

Diacylglycerol lipase (DAGL): enzyme with α and β isoforms, hydrolyzing 1,2-DAG to 2-MAG (including 2-AG) and fatty acids

3. BIOSYNTHESIS OF ENDOCANNABINOIDS AND ENDOCANNABINOID-LIKE MOLECULES IN MAMMALS

3.1. Biosynthesis of 2-AG and Other 2-MAGs

As an endocannabinoid, 2-AG is produced on demand by the receptor-mediated breakdown of membrane phospholipid precursors without storage in intracellular compartments (127). Despite structural and functional similarities, the biosynthetic pathways of 2-AG completely differ from those of AEA (137). 2-AG, which has been detected in various animal tissues, was recognized as a transitional metabolite produced by the hydrolytic cleavage of arachidonic acid–containing diacylglycerol (DAG) in the degradative pathways of glycerophospholipids (127). Although the arachidonic acid chain is abundant at the *sn*-2 position of glycerophospholipid molecules, other fatty acyl species also bind to the same position. Therefore, 2-MAGs other than 2-AG may be concomitantly produced in the same pathways. To date, several distinct biosynthetic pathways of 2-AG have been discovered (**Figure 2**). Arachidonic acid–containing inositol phospholipids may be the most important precursor for the production of 2-AG in the brain (128).

The primary pathway depends on the activity of phospholipase C (PLC) for the hydrolysis of phosphatidylinositol (PI) 4,5-bisphosphate to DAG and inositol trisphosphate. Mammalian PLC enzymes exist in 13 isozyme forms, which are classified into six subfamilies: β, γ, δ, ε, ζ, and η. Among them, β-type isozymes, each consisting of approximately 1,200 amino acids, have been analyzed most extensively (43, 53). Four isoforms (β_{1-4}) of PLC-β are composed of the domains common in all isozymes (the PH, EF, X, Y, and C2 domains) with the addition of approximately 400 amino acids. This activity is increased by the α subunit of the $G_{q/11}$ protein, which is activated in response to the hormonal stimulation of G protein-coupled receptors (53). Diacylglycerol lipase (DAGL) then facilitates the hydrolysis of the resultant DAG at the *sn*-1 position for the formation of 2-AG. Two isoforms (α and β) exist as the *sn*-1-specific DAGL in mammals (11). In humans, DAGLα (1,042 amino acids) is larger than DAGLβ (672 amino acids) with a carboxyl-terminal tail of 370 amino acids, but these isoforms share 33% sequence identity. Gene-deficient mice lacking DAGLα and -β showed significant loss of 2-AG in various tissues. For example, DAGLα deficiency caused reductions in 2-AG of approximately 80% and 60% in the brain and liver, respectively, whereas DAGLβ-deficient mice showed an approximately 50% reduction of 2-AG in the brain and as much as 90% in the liver (45). Therefore, both DAGLs were assumed to be responsible for the majority of 2-AG production in the brain, liver, and other tissues. DAGLs are currently emerging as therapeutic targets for a wide range of pathophysiological conditions, including fragile X syndrome, pain, inflammatory diseases, and obesity (125).

The second pathway consists of both phospholipase A_1 (PLA_1) and lysophosphatidylinositol-specific PLC (lysoPI-PLC), which differs from other types of PLC and is found in synaptosomes (136). In this pathway, PI was shown to be initially hydrolyzed by PLA_1, and the lysoPI

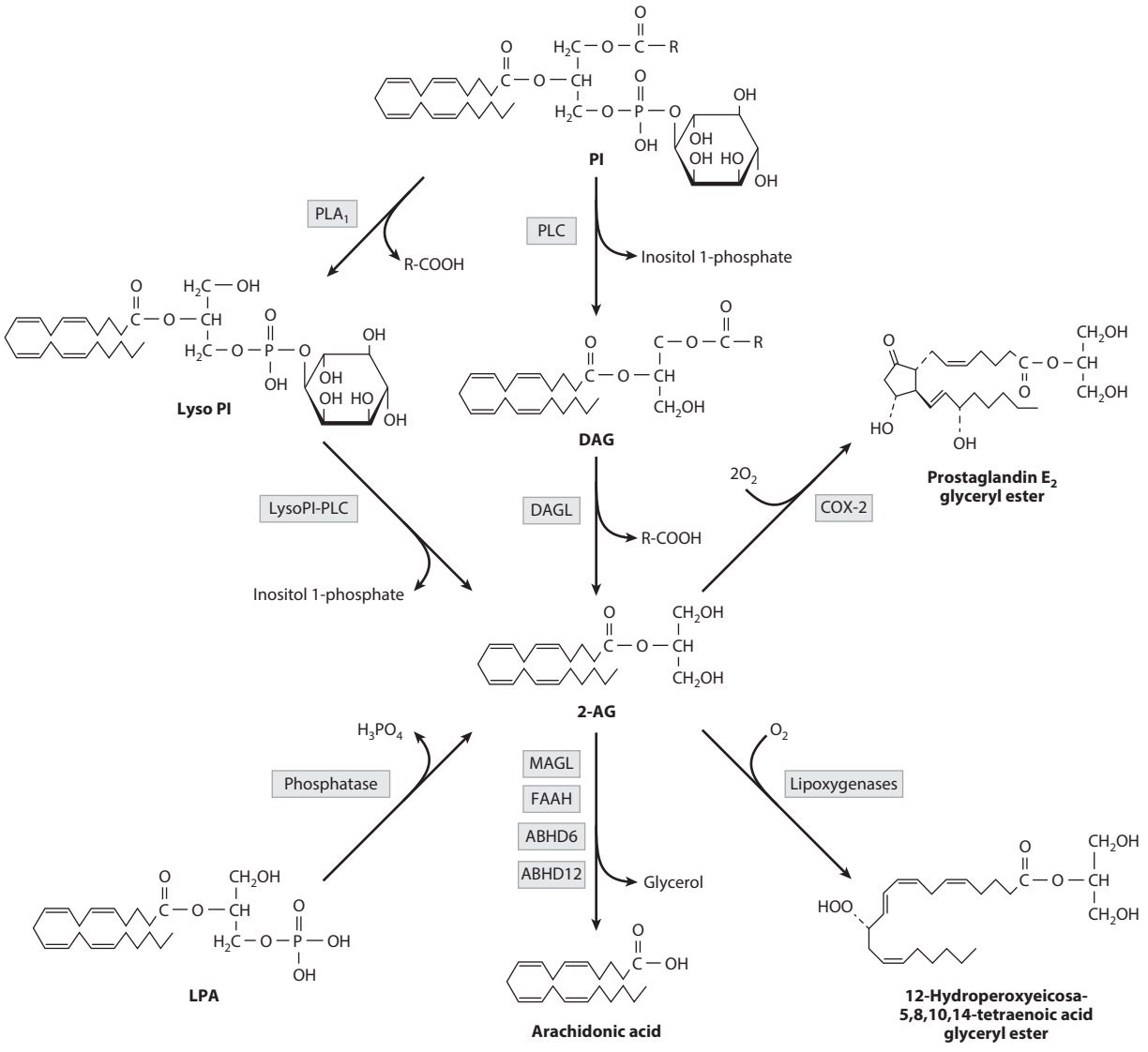


Figure 2

Metabolism of 2-AG, which is formed by hydrolysis of phospholipids and degraded by hydrolysis or oxygenation. Abbreviations: 2-AG, 2-arachidonoylglycerol; ABHD, α,β-hydrolase domain; COX-2, cyclooxygenase-2; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; LPA, lysophosphatidic acid; lysoPI-PLC, lysophosphatidylinositol-specific phospholipase C; MAGL, monoacylglycerol lipase; PLA_1, phospholipase A_1; PLC, phospholipase C.

produced served as a substrate for lysoPI-PLC, which was ultimately hydrolyzed to 2-AG (135). Alternatively, 2-AG may be produced in the rat brain from 2-arachidonoyl lysophosphatidic acid (LPA) by a phosphatase (99). Several types of glycerophospholipids other than PI were also shown to serve as precursors for the biosynthesis of 2-AG. For example, 2-AG was formed from 2-arachidonoyl phosphatidic acid (PA) in ionomycin-treated mouse neuroblastoma cells via 2-arachidonoyl DAG (12).

3.2. Biosynthesis of AEA and Other NAEs

Due to biochemical and pharmacological interests, the formation and breakdown of NAEs in mammalian tissues have been broadly analyzed (**Figure 3**). NAEs are stored in negligible amounts in intracellular compartments, and when needed, different NAEs, including AEA, PEA, and

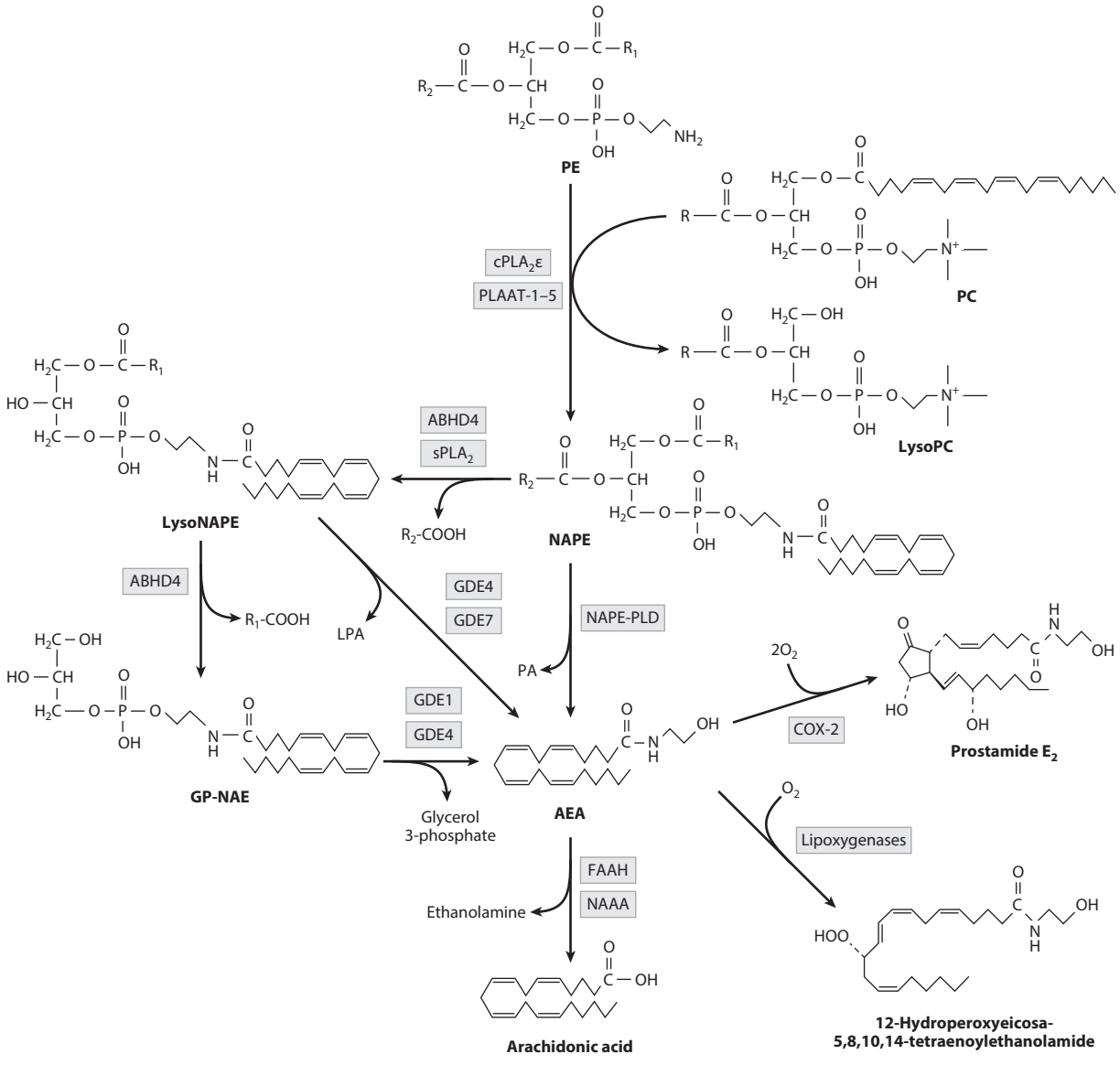


Figure 3

Metabolism of AEA, which is formed from phospholipids via NAPE and degraded by hydrolysis or oxygenation. Abbreviations: ABHD, α,β-hydrolase domain; AEA, arachidonoylethanolamide; COX-2, cyclooxygenase-2; cPLA$_2$ε, ε isoform of cytosolic phospholipase A$_2$; FAAH, fatty acid amide hydrolase; GDE, glycerophosphodiesterase; GP-NAE, glycerophospho-*N*-acylethanolamine; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; NAPE, *N*-acyl-phosphatidylethanolamine; PC, phosphatidylcholine; PLAAT, phospholipase A and acyltransferase; PLD, phospholipase D; sPLA$_2$, secretory phospholipase A$_2$.

OEA, are produced through common pathways (59, 138). Some membrane glycerophospholipids serve as precursors for their biosynthesis, and the canonical pathway consists of two sequential enzymatic steps. The first step is the transfer of a fatty acyl group from the *sn*-1 position of glycerophospholipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), to the amino group of PE, resulting in the formation of an *N*-acyl-phosphatidylethanolamine (NAPE). NAPEs are a unique class of glycerophospholipids with three acyl chains. This reaction is catalyzed by Ca^{2+}-dependent or -independent *N*-acyltransferases (59). The former enzyme was identified as the ε isoform of cytosolic phospholipase A_2 ($cPLA_2\varepsilon$ or PLA2G4E) (102), while the latter enzyme was shown to belong to the PLAAT (phospholipase A and acyltransferase) family. PLAATs were originally found to function as tumor suppressors but were subsequently shown to exhibit enzyme activities toward glycerophospholipids (59). In living cells, $cPLA_2\varepsilon$ is activated in response to an increase in intracellular Ca^{2+} concentrations, which may be caused by cell injury or receptor-mediated signals (98). On the other hand, PLAATs may maintain the basal levels of NAPE under steady-state conditions without specific stimuli. Plasmenylethanolamine (the ethanolamine phospholipid of the plasmalogen type) was also used as an acyl acceptor substrate of *N*-acyltransferase, and the resultant *N*-acyl-plasmenylethanolamine (plasmalogen-type NAPE) was detected in mouse brain (134).

N-acyl-phosphatidylethanolamine (NAPE): class of triacylated phospholipids, serving as a precursor of bioactive NAEs

cPLA$_2\varepsilon$: an isoform of cytosolic phospholipase A_2 enzymes, also called PLA2G4E, functioning as Ca^{2+}-dependent *N*-acyltransferase to form NAPE from phosphatidylethanolamine

NAPE-hydrolyzing phospholipase D (NAPE-PLD): an enzyme that specifically hydrolyzes NAPEs to phosphatidic acid and NAEs, including AEA, PEA, and OEA, in mammals

The second step is the hydrolysis of NAPE to NAE and PA, which is catalyzed by NAPE-hydrolyzing phospholipase D (NAPE-PLD). This enzyme belongs to the zinc metallo-hydrolase family of the β-lactamase fold, and its protein structure differs from those of typical PLDs (103). The enzyme specifically hydrolyzes NAPEs with a long- or medium-chain fatty acyl group at the *N* position but is inactive with common glycerophospholipids, such as PC and PE. Although NAPE-PLD activity is enhanced in the presence of high concentrations of divalent cations, such as Mg^{2+} and Ca^{2+}, it is unclear whether this activity is regulated by receptor-mediated signals. Since NAPE-PLD-deficient mice still produced NAEs, the presence of supplementary pathways for NAE formation became clear (124, 129, 134, 139). In the brain and other tissues of NAPE-PLD-deficient mice, endogenous levels of *N*-acyl-lysoPE (lysoNAPE) and glycerophospho-*N*-acylethanolamine (GP-NAE) were markedly increased, and these molecules were considered intermediate metabolites in NAPE-PLD-independent multistep pathways for the formation of NAE (**Figure 3**). Subsequent studies identified several enzymes involved in these pathways, which included α,β-hydrolase domain containing 4 (ABHD4) and GDE1, -4, and -7, members of the glycerophosphodiesterase (GDE) family (59). It remains unclear why multiple pathways exist in the body to produce NAEs from NAPEs.

AEA is a minor component of NAEs in most animal tissues, and all of the aforementioned pathways appear to preferentially generate saturated and monounsaturated NAEs, including PEA and OEA, rather than polyunsaturated NAEs, such as AEA. The main reason may be that $cPLA_2\varepsilon$ transfers an acyl chain exclusively from the *sn*-1 position of glycerophospholipids, at which saturated and monounsaturated acyl groups are favorably integrated (102), and that the majority of the resultant NAPEs have a saturated or monounsaturated acyl group at the *N* position (134). Recombinant PLAAT members also produce these NAPEs from endogenous phospholipids when overexpressed in mammalian cells (139). Apart from these pathways via NAPE, NAEs may also be formed by the condensation of free fatty acids with ethanolamine in the reverse reaction of FAAH when both substrates are present in sufficient amounts (73). Since arachidonic acid is reported to be a more favorable substrate than other fatty acids in this reaction, some AEA may be formed in vivo by this route (107).

4. DEGRADATION OF ENDOCANNABINOIDS AND ENDOCANNABINOID-LIKE MOLECULES IN MAMMALS

4.1. Degradation of 2-AG and Other 2-MAGs

Monoacylglycerol lipase (MAGL): a principal enzyme hydrolyzing 2-AG and other MAGs to free fatty acids and glycerol

The production of 2-AG has numerous biological impacts on tissues and cells. The accumulation process of 2-AG in cells was presumed to be controlled by rapid transport through a membrane transporter that is similar to the anandamide membrane transporter for the transportation of NAEs (4). 2-AG is then hydrolyzed to free arachidonic acid and glycerol by several hydrolases. Monoacylglycerol lipase (MAGL) is the most important enzyme for the hydrolysis of 2-AG and possibly other MAGs (13).

MAGL is a typical serine hydrolase with the catalytic triad of Ser-Asp-His and belongs to the α/β hydrolase superfamily (119). This enzyme is involved in lipolysis as well as in the regulation of the endocannabinoid system (49). The primary structure of mouse MAGL comprises 302 amino acids with a molecular mass of 33.2 kDa, whereas its human counterpart has 303 amino acids and a molecular mass of 33.4 kDa (119). The three-dimensional structure of human MAGL revealed the formation of a homodimer. Regarding the secondary structure, both the α/β hydrolase fold and the lid domain are necessary for catalytic activity (74). MAGL is distributed in a wide range of tissues including the brain, liver, adipose tissue, and intestines. Brain MAGL was shown to be expressed in neurons, astrocytes, oligodendrocytes, and microglia. Concerning substrate specificity, MAGL preferentially acts on MAGs over TAGs and DAGs but does not distinguish between 1(3)-AG and 2-AG. The enzyme may hydrolyze a wide variety of saturated and unsaturated acyl chains of both medium- and long-chain fatty acids with a preference for unsaturated acyl chains (74). Numerous chemotypes have been reported to date as irreversible or reversible inhibitors of MAGL activity. Maleimides, disulfides, carbamates, ureas, and arylthicarmide are considered irreversible inhibitors, whereas tetrahydrolipstatin-based derivatives, isothiazolines, natural terpenoids, and some amide-based derivatives have been used as reversible inhibitors (25). Specific MAG inhibitors, which increase endogenous 2-AG levels, are expected to exert pharmacological effects on a number of disease states, including pain, inflammation, metabolic disorders, neurodegenerative pathologies, anxiety, epilepsy, and cancer (25).

Although ABHD6, ABHD12, and FAAH may also hydrolyze 2-AG, they are not the main enzymes degrading 2-AG. Cravatt and coworkers showed that MAGL accounted for approximately 85% of total 2-AG-hydrolyzing activity in the brain, whereas ABHD6 and ABHD12 contributed approximately 4% and 9%, respectively (14). FAAH, a principal enzyme for the hydrolysis of NAEs, was identified as the fourth contributor for the hydrolysis of 2-AG, contributing approximately 1%. The administration of URB597, a FAAH inhibitor, increased 2-AG levels in the mouse brain (127, 137). Alternatively, some anabolic enzymes may integrate 2-AG into complex lipid molecules. These enzymes include MAG kinase, which phosphorylates 2-AG to generate 2-arachidonoyl-LPA, and MAG acyltransferase, which catalyzes the formation of DAG from MAG (65, 121). 2-Arachidonoyl-LPA may be further converted into 1-stearoyl-2-arachidonoyl-PA and may enter the PI cycle or be used in the biosynthesis of phospholipids (127).

Similar to free arachidonic acid, the arachidonoyl chain of 2-AG is oxygenated by both cyclooxygenase-2 (COX-2) and lipoxygenases in mammalian tissues, leading to the production of glyceryl prostaglandins and hydroperoxy derivatives, respectively (**Figure 2**) (71, 117, 137). Although the physiological significance of oxygenated derivatives, such as glyceryl prostaglandins, remains unclear, biological activities including ligand activity for PPAR-α have been reported (117). Since glyceryl prostaglandins exhibited longer half-lives than usual prostaglandins (70), it was assumed that they act as a type of systemic mediator or prodrug when they are transferred from their production sites to the target tissues (71).

4.2. Degradation of AEA and Other NAEs

N-acylethanolamine-hydrolyzing acid amidase (NAAA): a lysosomal enzyme abundant in macrophages, hydrolyzing various NAEs with PEA being the most reactive substrate under acidic conditions

In a rapid deactivation process, AEA is thought to be taken up inside cells by anandamide membrane transporters, which have not yet been identified (4). AEA may interact with compartments of the membrane or with intracellular binding proteins for its effective accumulation and subsequent uptake by the caveolae-mediated endocytic process (4). The storage of AEA in lipid droplets has also been reported and represents an active reservoir for the segregation of AEA (61). AEA was shown to be transported from the plasma membrane to the site of FAAH for its degradation, which was facilitated by fatty acid–binding proteins (62). Maccarrone and coworkers also identified heat-shock protein 70 as a cytosolic AEA-binding protein (101).

To date, FAAH (also referred to as FAAH-1 to distinguish between two isozymes) and *N*-acylethanolamine-hydrolyzing acid amidase (NAAA) have been characterized as the two most important enzymes responsible for the hydrolysis of NAEs (**Figure 3**). FAAH is a serine hydrolase with Ser-Ser-Lys as a catalytic triad and belongs to the amidase signature family (22). The enzyme hydrolyzes various bioactive NAEs, including AEA, OEA, and PEA, and is ubiquitously distributed in mammalian tissues with high expression levels in the brain and liver. FAAH-2, a second isozyme with an amino acid sequence identity of approximately 20%, was also found in humans, but not in rodents, and was reported to exhibit similar enzyme activities (145). FAAH-1 and -2 are located on the endoplasmic reticulum and cytoplasmic lipid droplets, respectively (13, 61, 138). Numerous specific FAAH inhibitors have been developed, which have potential as therapeutic drugs for the treatment of chronic pain, metabolic disorders, psychoses, nausea and vomiting, depression, anxiety disorders, and some phobias (21, 87, 133).

NAAA is a lysosomal cysteine hydrolase that is abundantly expressed in macrophages and hydrolyzes various NAEs, with PEA being the most reactive substrate (139). Its optimal pH of 4.5–5 makes it suitable for functioning in lysosomes. Due to the analgesic and anti-inflammatory effects of PEA as well as the high expression level of NAAA in macrophages, specific NAAA inhibitors are expected to be effective for various types of pain, including thermal hyperalgesia and mechanical allodynia (3). To discover specific NAAA inhibitors, researchers either utilized PEA for chemical modulation or targeted the catalytic cysteine for covalent modifications. The latter strategy enabled development of different types of potent inhibitors, which included β-lactone derivatives, such as URB913 and ARN0772, and 2-naphthamide (112). These NAAA inhibitors were reported to exert beneficial effects against lung inflammation, dermatitis, spinal cord trauma, inflammatory bowel disease, and some types of pain. However, none of the NAAA-targeting inhibitors have yet reached the clinical trial stage (52, 86).

The complex biochemical frameworks of NAE metabolism also include other enzymes and products. Similar to 2-AG, the arachidonic acid chain of AEA may be exposed to oxygenation with the aid of COX-2 and lipoxygenases alongside several cytochrome P450 monooxygenases (**Figure 3**) (128, 147). COX-2 turns AEA into prostaglandin-ethanolamides (prostamides) and lipoxygenases into diverse hydroperoxyeicosatetraenoyl-ethanolamides. Epoxyeicosatrienoyl-ethanolamides and hydroxyeicosatetraenoyl-ethanolamides were produced from AEA by cytochrome P450 (83). Other polyunsaturated NAEs may also be oxygenated to form ethanolamide oxylipins with bioactivities such as vasomodulatory effects (72, 113).

5. ROLES OF ENDOCANNABINOIDS IN ENERGY HOMEOSTASIS AND METABOLIC REGULATION

5.1. Central Nervous System Endocannabinoids That Control Feeding Behaviors

Cannabis, a plant that produces marijuana, specifically increases appetite, which results in considerable weight gain (1). The regulation of feeding behavior and energy homeostasis is associated

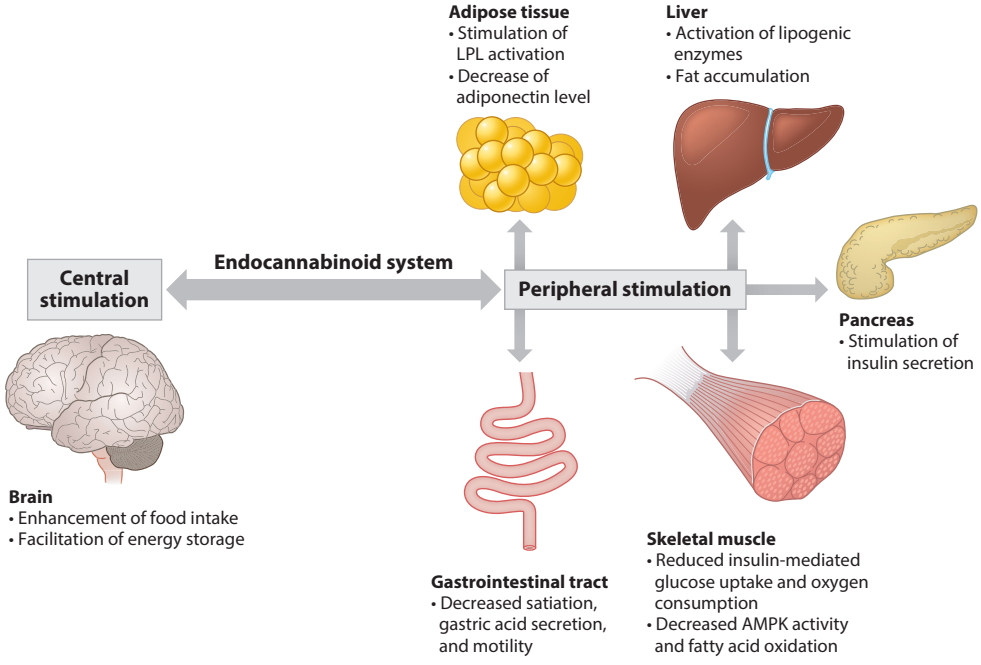


Figure 4

Biological functions of the endocannabinoid system through CB1 in central and peripheral tissues. Abbreviations: AMPK, adenosine monophosphate–activated protein kinase; CB1, central-type cannabinoid receptor; LPL, lipoprotein lipase.

with the endocannabinoid system, resulting in the modulation of central nervous system (CNS) signaling processes (**Figure 4**). As discussed above, AEA acts as a partial CB1 agonist and a weak CB2 agonist, while 2-AG is a full agonist of both receptors. CB1 is one of the most abundant receptors in many brain regions, including the prefrontal cortex, globus pallidus, substantia nigra, hippocampus, striatum, and molecular layer of the cerebellum (77), and the stimulation of feeding behavior by the administration of CB1 agonists in the CNS is now well established. The antagonism of CB1, but not CB2, successfully attenuated the stimulatory effects of agonists on food intake (146). The first selective CB1 antagonist to be discovered, rimonabant (also known as SR141716), was shown to decrease food consumption during the appetitive and consumption phases of feeding behavior but did not block the Pavlovian response for a palatable stimulus (38). In animal studies, CB1-deficient mice ate a smaller amount than their wild-type littermates. Food intake by these mice was unaffected by a treatment with rimonabant (132).

Rimonabant: also known as SR141716, a specific CB1 antagonist that had been approved as an antiobesity drug in European countries but was quickly withdrawn due to side effects

To deal with these complex processes, the CNS and peripheral tissues have to be synchronized. Mesolimbic and hypothalamic regions have been identified as the main brain areas to stimulate food intake. The endocannabinoid system is expressed in mammalian brain structures that modulate the hypothalamic control of food intake (7). In hypothalamic CB1-deficient mice, energy expenditure was increased through the stimulation of β_3-adrenergic receptors and uncoupling protein-1 in brown adipose tissue (BAT), resulting in decreased body weight gain (19). The hypothalamus was presumed to be responsible for inhibiting AEA synthesis in adipose tissue through sympathetic activation, which inhibits lipogenesis. This finding suggested the importance of the peripheral nervous system through the engagement of the endocannabinoid system between the CNS and peripheral organs (116).

Additionally, the hypothalamus accepts some inputs from peripheral hormones, which notify it of peripheral energy status. Among peripheral hormones, leptin exerts anorexigenic effects, whereas ghrelin and glucocorticoid exert orexigenic effects. Changes in hypothalamic endocannabinoid levels inversely correlated with the serum levels of leptin. Therefore, leptin was suggested to decrease endocannabinoid levels in the hypothalamus by anorexigenic signaling (29). Another gut hormone, ghrelin, also appeared to be synergistically involved with endocannabinoids in the hypothalamus for increasing appetite levels to promote food intake (116). Circulating ghrelin levels were elevated in response to food deprivation, which ultimately increased the hypothalamic levels of endocannabinoids. The systemic administration of rimonabant decreased the circulating levels of ghrelin and, thus, reduced food intake (7). Conversely, glucocorticoids suppressed glutamatergic excitatory synapses to inhibit hypothalamic neurons, and this mechanism was mediated through the retrograde release of endocannabinoid and CB1 activation (116).

Another crucial brain region involved in eating behavior is the reward system, which consists of a group of synaptically interconnected neural structures. This system is responsible for the observed increase in eating behavior in response to the induction of pleasant sensations (10). Supporting a hypothetical connection between the endocannabinoid and reward systems, fasting increased endocannabinoid levels in the nucleus accumbens, which is involved in the reward system. Di Marzo and coworkers (68) showed that fasting increased AEA and 2-AG levels in the limbic forebrain but only slightly elevated 2-AG levels in the hypothalamus region. The direct administration of 2-AG to the nucleus accumbens shell enhanced food intake in a CB1-dependent manner (68). The reward system was recently reported to be directly suppressed by insulin with the help of local endocannabinoids (116). The nucleus of the tractus solitarius is also involved in feeding, as it recognizes sensory inputs from the nodose ganglion. CB1 was expressed in both of these regions, suggesting the involvement of endocannabinoids in the modulation of food intake by this circuit (116).

Therefore, the overstimulation of the endocannabinoid system may be involved in overeating and obesity. NAEs and 2-AG were purified from various natural food sources of both animal origin (human, bovine, and goat milk) and plant origin (cacao powder, different herbs, and tea) (31, 46). However, the content of NAEs and oleamide from cacao powder was not sufficient to exert cannabis-like effects, and the presence of 2-AG and oleamide in milk samples needs further investigation. Several herbs and teas contained compounds that may enhance the effects of endocannabinoids, such as β-caryophyllene, which is a terpene available in black pepper, oregano, cinnamon, clove, and many other herbs (46). β-Caryophyllene selectively stimulated CB2 to improve the immune system during infections. Kaempferol, which is present in *Camelia sinensis*—commonly known as tea—prevented the breakdown of AEA by inhibiting FAAH. Epigallocatechin gallate, the most abundant catechin in tea, was found to stimulate CB1 at a very small amount (92). Curcumin, contained in turmeric, increased endocannabinoid levels (92).

5.2. Endocannabinoids in Peripheral Organs for Metabolic Regulation

The endocannabinoid system also plays important roles in metabolically relevant peripheral tissues, including adipose tissue, liver, muscle, and the endocrine pancreas through the peripheral nervous system and hormones (**Figure 4**) (116). White adipose tissue (WAT) functions not only as a major energy repository organ, but also as an endocrine organ that releases adipokines to regulate energy homeostasis. The presence of CB1 in mature white adipocytes in both humans and rodents indicated the involvement of AEA and 2-AG in WAT-induced energy homeostasis mechanisms (122). Subsequently, human white subcutaneous adipocytes were found to produce measurable amounts of AEA and 2-AG as well as related molecules, such as PEA and OEA. The subcutaneous

and visceral adipocytes of both humans and rats also expressed FAAH and MAGL, which was confirmed by radiolabeled substrates. As discussed below, there is clear evidence for the involvement of the endocannabinoid system in the regulation of both adipogenesis and lipogenesis (88).

The stimulatory effects of both AEA and 2-AG via CB1 on adipocytes may affect lipid metabolism through the enhancement of fatty acid biosynthesis as well as the accumulation of TAG (141). The overall mechanism includes reductions in adiponectin levels and AMP-activated protein kinase (AMPK) expression as well as the stimulation of lipoprotein lipase activity and PPAR-γ expression (**Table 1**). A reduced level of AMPK was shown to increase fatty acid synthesis but decrease fatty acid β-oxidation (123). Experiments with adipocyte-specific CB1-deficient mice showed that these findings were more prominent in males than in females, indicating that endocannabinoid regulation in WAT may be sensitive to sex hormones (142). Functional CB1 has also been detected in BAT, which suggests a role for CB1 in reducing energy expenditure via decreased thermogenesis in obese mice (122). CB1 was upregulated just before adipocyte differentiation, which increased the expression levels of the adipogenic enzymes responsible for the synthesis of fatty acids and TAG in adipose tissue. Endocannabinoid levels also rapidly increased before adipocyte differentiation, and leptin then promptly reduced the levels of AEA and 2-AG produced by mature adipocytes (89, 116). Moreover, leptin suppressed AEA and 2-AG levels in mouse 3T3-F442A adipocytes (80). CB1 antagonism activated BAT thermogenesis and glucose uptake with the coordination of a complete sympathetic nervous system. This antagonism also caused an increase in glycolysis and insulin sensitivity, which was independent of its suppressive effects on food intake (2).

Signals to the CNS from the small intestine and other organs of the gastrointestinal tract play a crucial role in the regulation of energy balance with the coordination of the endocannabinoid system (30). CB1 and CB2 are both expressed in the gastrointestinal tract and involved in gut–brain communication. AEA and 2-AG interfered with the total process in both the endocrine and neural pathways through the activation of CB1. The activation of CB1 modulated nutrient processing by inhibiting gastric juice secretion or discharge and intestinal motility with the help of intestinal hormones (144). Ghrelin and endocannabinoids act synergistically to promote food intake and hunger by triggering nerve impulses to the hindbrain and hypothalamus via blood (105, 144). After 24 h of fasting, AEA levels in the small intestine were sevenfold higher than those in the nonfasting group, suggesting that endocannabinoid levels are reactive to the nutrient status (51). Collectively, these findings demonstrate that the endocannabinoid system may exert important effects in the gastrointestinal tract by both endocrine and neural pathways.

Liver also plays key roles in regulating energy homeostasis and metabolism. Hepatocytes express CB1 and produce endocannabinoids, which may affect glucose metabolism as well as the development of fatty liver disease and cirrhosis (5). Under hepatosteatosis conditions induced by a high-fat diet in mouse models, the expression levels of CB1 and the levels of 2-AG and AEA were significantly increased in the liver (60, 104). These changes were also detected in patients with hepatosteatosis (122). The enhancement of the endocannabinoid system disrupted hepatic lipogenic and lipolytic processes as well as insulin signaling pathways, which resulted in fatty liver diseases (**Table 1**). Fatty liver caused by a high-fat diet has also been linked to insulin resistance, characterized by an increase in hepatic glucose production, plasma hyperglycemia, and hyperinsulinemia. The administration of AEA and arachidonyl-2′-chloroethylamide in male Wistar rats revealed that the activation of CB1 correlated with the induction of glucose intolerance. In contrast, the CB1 receptor antagonist AM251 exerted the opposite effects (9). CB1-deficient mice still developed diet-induced obesity but were resistant to hepatosteatosis and insulin insensitivity (122). Furthermore, the acute application of isopropyl dodecylfluoro-phosphonate in mice, which

increased 2-AG and AEA levels by inhibiting both MAGL and FAAH, resulted in decreased glucose tolerance, which directly emphasized the effects of hepatic CB1 on insulin sensitivity (118).

Muscle is a crucial tissue in the regulation of energy homeostasis through β-oxidation and glucose mobilization, which occur not only with exertion but also during rest (122). It strongly expresses CB1, CB2, and TRPV1 as well as some endocannabinoid-related enzymes, including FAAH and MAGL (20, 23). In genetically obese female Zucker rats, the expression levels of CB1 mRNA in the soleus muscle were lower than those in lean female rats, whereas AEA levels were higher (78). However, the opposite was observed in the soleus muscle of mice with obesity induced by a high-fat diet; the expression levels of CB1 were increased (122). The soleus muscle of obese mice showed elevated levels of 2-AG, but not AEA, after 3 weeks of a high-fat diet treatment as well as at the end (after 14 weeks) of treatment, but not in the interim period (90). The chronic treatment of leptin-deficient mice and insulin-sensitive obese or lean Zucker rats with rimonabant elevated glucose uptake in the soleus muscle, while AEA significantly decreased glucose uptake (78, 79). Furthermore, the endocannabinoid system modified other insulin signaling pathways, in which higher doses of AEA caused the activation of different types of protein kinases, thereby inhibiting insulin-dependent glucose uptake in human skeletal muscle (**Table 1**) (32).

The endocrine pancreas also contains a complete endocannabinoid system that appears to be involved in insulin secretion and β-cell division. Pancreatic islets have both CB1 and CB2 as well as other machinery of the endocannabinoid system; however, it remains unclear whether β-cells express CB1 (116). The activation of CB1 and CB2 by AEA and 2-AG in human pancreatic islets enhanced the secretion of insulin, whereas only 2-AG increased glucagon secretion. These effects of CB1 appeared to be mediated by alterations in glucose-induced intracellular calcium transients. However, the activation of CB2 with specific agonists lowered insulin secretion, which was not linked to CB2-induced somatostatin release (8). The endocannabinoid system also regulates glucose metabolism via pancreatic hormonal discharge that favors glucose uptake in the liver for fatty acid synthesis and energy storage (**Table 1**). Therefore, from a physiological perspective, the signaling process of CB1 exerts anabolic effects through food intake, lipid metabolism, and glucose metabolism in different organs and tissues (116).

Enhanced endocannabinoid signaling in obese people may be involved in the pathophysiology of obesity. CB1 was found to be upregulated in the liver and adipose tissues of both obese humans and obese mice. CB1-deficient mice were completely resistant to obesity induced by a high-fat diet. Moreover, the blockade of CB1 suppressed this overactivity of the endocannabinoid system as well as the progression of obesity (122). The involvement of endocannabinoids in the progression of human obesity is supported by the following findings. Circulating AEA and 2-AG levels were found to be higher in obese individuals and appeared to positively correlate with free fatty acid and TAG levels (80). A reduction in the visceral fat mass in overweight men was related to a decrease in blood plasma 2-AG levels (28). The P129T variant of FAAH was more frequently detected in overweight and obese individuals (126). Additionally, an overactive endocannabinoid system was found to be associated with fatty liver due to increased hepatic lipogenesis, which ultimately led to nonalcoholic steatohepatitis (100). However, the key regulatory factors of the endocannabinoid system involved in the progression of obesity have not yet been identified.

6. ROLES OF ENDOCANNABINOID-LIKE MOLECULES IN ENERGY HOMEOSTASIS AND METABOLIC REGULATION

6.1. Roles of OEA and Other NAEs

Among endocannabinoid-like molecules, OEA appears to be the most important molecule in the regulation of appetite, fat metabolism, and energy homeostasis, suggesting its therapeutic

potential in the management of the obesity pandemic (**Table 1**). The biological effects of OEA are mediated through several molecular targets in the gut–brain axis, which include receptors such as PPAR-α, GPR119, TRPV1, and CD36 (also known as fatty acid translocase) and neurotransmitters including dopamine and histamine, along with the neuropeptide oxytocin (48).

OEA-mediated appetite suppression or satiety was initially observed in Wistar rats by the research group of Piomelli (24). This observation was followed by the identification of OEA's primary receptor, PPAR-α (EC_{50}, 0.12 μM) (40). PPAR-α-deficient mice did not show the hypophagic effects of OEA, suggesting its principal role in satiety (40). OEA levels in the rat jejunum were decreased by fasting and increased by refeeding (24). Among diet compositions, fat increased OEA levels, while sugar and protein had no effect (33). Among lipid substances, oleic acid, but not palmitic acid, was responsible for this upregulation of OEA by stimulating the biosynthesis of *N*-oleoyl-PE, the precursor of OEA (33). CD36 expressed in intestinal cells was subsequently shown to be crucial for the uptake of oleic acid for OEA biosynthesis because CD36 gene-disrupted mice failed to produce OEA upon feeding (54). A recent clinical trial in which 56 obese subjects were supplemented with 250 mg of OEA for 8 weeks showed the upregulation of the PPAR-α gene (2.41-fold) in peripheral blood mononuclear cells with a significant reduction in body weight (75). This clinical outcome was consistent with previous findings showing the upregulation of CD36 and lipid-oxidizing enzymes as major downstream targets of PPAR-α (42). In addition to appetite suppression and increased lipolysis in adipocytes, OEA was also suggested to enhance fatty acid uptake in enterocytes via the increased expression of CD36 (149). However, the chronic intake of a fatty diet reduced OEA levels in rodent guts, resulting in overeating and obesity. This finding was later linked to the observation of diminished brain dopaminergic function in obese mice, and their feeding behavior was corrected by the administration of exogenous OEA (131). In addition, extrahepatic mast cell–derived histamine was reported to stimulate the formation of OEA in the liver via the H_1-receptor, and this OEA was found to play a key role in fasting-induced ketogenesis (95).

Since NAPE-PLD catalyzes the release of NAEs, including OEA, from precursor NAPEs (103), its involvement in obesity has been investigated in various studies. For example, NAPE-PLD expression was reduced in the jejunum upon fasting and increased after refeeding (39). The overexpression of NAPE-PLD in the rat proximal small intestine, effected by injecting a NAPE-PLD-harboring viral vector, caused a local increase in OEA as expected, resulting in lower food intake (41). In contrast, mice with the inducible deletion of the NAPE-PLD gene specifically in intestinal epithelial cells developed hyperphagia when fed a high-fat diet, resulting in obesity and steatosis (34). These findings suggested that NAPE-PLD is responsible for the local generation of OEA in the upper small intestine. Mice with conditional NAPE-PLD deficiency specific for adipose cells showed reduced formation of OEA in this tissue with aberrant lipid metabolism, leading to an increase in body weight (47). OEA also increased the level of peptide YY (PYY), an appetite-reducing hormone (108). Therefore, OEA is a proven dynamic signaling molecule that maintains energy homeostasis under both fasting and well-fed conditions by targeting receptors other than CB1 and has potential as a pharmacological substitute for anorexiants and antiobesity drugs without their notorious side effects through CB1. The anorexic and antiobesity effects of OEA were not as strongly associated with stress and malaise as rimonabant (115).

The physiological mechanisms by which OEA confers satiety at the molecular level remain unclear. Gut-derived OEA is considered to provide satiety by increasing pleasure-associated molecules, such as dopamine (131) and oxytocin (17), in the brain, at least in rodents. However, further studies are needed to confirm this gut–brain axis relationship. Moreover, it currently remains unclear whether a diet rich in oleic acid increases OEA in humans; further research is warranted.

In addition, more clinical trials are required to establish a safe dosage of OEA supplementation for the management of obesity.

In addition to PPAR-α, the cell surface receptors GPR119 and TRPV1 were also reported to act as receptors for OEA (111). Once stimulated by OEA, GPR119, expressed in gut L-cells, secreted glucagon-like peptide (GLP)-1, which inhibited glucagon secretion with the concurrent stimulation of insulin secretion by sugar. Other biological effects of GLP-1 include a decrease in gastric emptying and the inhibition of food intake, including the postmeal reward sensation. GLP-1 receptor agonists are already in clinical use for the treatment of type 2 diabetes, including some ongoing clinical trials for the treatment of obesity (97). On the other hand, TRPV1 is generally known as a capsaicin receptor, which is responsible for the perception of heat and pain. However, the appetite-suppressing effect of OEA was not abolished in mice deficient in GPR119 or TRPV1, suggesting that these receptors are not essential for OEA-mediated satiety (58, 76).

Two other NAEs, PEA and LEA, also induced a rapid decrease in food intake when orally or intraperitoneally administered to rodents, and the anorectic effects of these NAEs were suggested to be mediated by their activation of PPAR-α (55). Although LEA showed a less potent anorectic effect than OEA, the endogenous level of LEA in the rodent jejunum was occasionally more than double those of OEA and PEA, suggesting its significant role in reducing food intake (55).

6.2. Role of 2-OG

Due to its structural similarity to OEA, 2-OG acts as an agonist of GPR119 to release GLP-1 with EC_{50} of 2.5 μM, but less potently than OEA (EC_{50}, 0.20 μM) (57). Nevertheless, the 2-OG-mediated production of GLP-1 through GPR119 appeared to be physiologically more important than OEA, evidenced by a very high ratio of 2-OG/OEA production after a fatty meal (**Table 1**). For example, the digestion of 5 mL of olive oil with pancreatic lipase produced approximately 2 g of 2-OG and 3.2 g of oleic acid (57). Therefore, 2-OG may reach millimolar concentrations in the gut during fat digestion, where it may serve as a local mediator releasing GLP-1 from ileal L cells (56).

7. CONCLUSIONS

Since the identification of cannabinoid receptors and endocannabinoids in the early 1990s, countless studies have been performed to elucidate the physiological significance of the endocannabinoid system. In parallel, the biological activities of saturated or monounsaturated NAEs and MAGs, introduced herein as endocannabinoid-like molecules, were also examined and found to be mostly mediated through other receptors, such as PPAR-α. Since obesity is a serious health issue worldwide, the effects of endocannabinoids and related molecules on feeding behavior and energy metabolism are important. Endocannabinoids stimulate appetite and increase body weight, while OEA exerts the opposite effects. Among endocannabinoids, 2-AG has been established as a physiologically true agonist of both CB1 and CB2, whereas AEA is presumed to be a minor by-product in the biosynthesis of abundant amounts of NAE species. Although locally accumulating AEA appears to exhibit some cannabimimetic activities, OEA and PEA as major NAEs may be physiologically more important than AEA. Therefore, further studies are needed to elucidate the molecular mechanisms underlying their biological activities as well as the development of NAE-related substances as therapeutic drugs against obesity, inflammation, and other disease states. Among MAGs, 2-OG is a major metabolite in the digestion of dietary fat, and its activity as a lipid mediator warrants further study.

FUTURE ISSUES

1. How important are NAEs, such as OEA and PEA, as physiological agonists of PPAR-α, compared to other endogenous ligands, including free fatty acids and eicosanoids?
2. What are the molecular mechanisms underlying appetite suppression by OEA?
3. From a long-range point of view, how does the dietary intake of ω3 and ω6 polyunsaturated fatty acids affect the in vivo effects of endocannabinoids and endocannabinoid-like molecules?
4. To what extent is AEA actually involved in the endocannabinoid system in comparison with 2-AG?
5. Apart from 2-AG, which has been established as an endocannabinoid, do other MAGs, including 2-OG, play physiological roles as lipid mediators?
6. Large amounts of NAPE and NAE accumulate during brain ischemia and heart infarction. What is the significance of this accumulation?
7. LysoNAPE and GP-NAE are intermediate metabolites in multistep pathways for the formation of NAE from NAPE. Do these molecules exhibit any biological activities?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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