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# Inactivation and Biotransformation of the Endogenous Cannabinoids Anandamide and 2-Arachidonoylglycerol

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2-AG: 2-arachidonoylglycerol

AEA: anandamide

CB1: cannabinoid receptor 1

CB2: cannabinoid receptor 2

PPAR: peroxisome proliferator-activated receptor

FAAH: fatty acid amide hydrolase

MAGL: monoacylglycerol lipase

COX: cyclooxygenase

LOX: lipoxygenase

PG-EAs: prostaglandin-ethanolamides

PG-GEs: prostaglandin-glycerol esters

HETE-EA: hydroperoxyeicosatetraenoylethanolamide

HETE-GE: hydroperoxyeicosatetraenoic acid glycerol ester

SERI: selective endocannabinoid reuptake inhibitor

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*Abstract*

The cannabinoid field is currently an active research area. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the most characterized endogenous cannabinoids (also known as endocannabinoids). These neuromodulators have been implicated in various physiologically-relevant phenomena including mood (Witkin *et al.*, 2005), the immune response (Ashton, 2007), appetite (Kirkham and Tucci, 2006), reproduction (Wang *et al.*, 2006), spasticity (Pertwee, 2002), and pain (Hohmann and Suplita, 2006). Pharmacological manipulation of AEA and 2-AG signaling should prove to have significant therapeutic applications in disorders linked to endocannabinoid signaling. One way to alter endocannabinoid signaling is to regulate the events responsible for termination of the endocannabinoid signal – cellular uptake and metabolism. However, in order to pharmacologically exploit AEA and/or 2-AG signaling in this way, we must first gain a better understanding of the proteins and mechanisms governing these processes. This review serves as an introduction to the endocannabinoid system with an emphasis on the proteins and events responsible for the termination of AEA and 2-AG signaling.

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## Endocannabinoid Signaling

Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the two most characterized members of the endocannabinoid family. AEA and 2-AG act as agonists for both intracellularly and extracellularly-localized receptors. Following on-demand biosynthesis, AEA and 2-AG serve as agonists for the G protein-

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coupled cannabinoid receptors CB1 and CB2 as well as the nuclear peroxisome proliferator-activated receptor (PPAR) family members PPARα and PPARγ (Felder *et al.*, 1993;Munro *et al.*, 1993;O'Sullivan, 2007). AEA is also an endogenous agonist for the vanilloid receptor channel TRPV1 (Zygmunt *et al.*, 1999;Smart *et al.*, 2000) and the GRP55 receptor (Pertwee, 2002;Ryberg *et al.*, 2007;Lauckner *et al.*, 2008). Cessation of AEA and 2-AG signaling occurs via a two-step process: 1) transport of endocannabinoids from the extracellular to the intracellular space, and 2) intracellular degradation by hydrolysis or oxidation.

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## Cellular Accumulation as a Mechanism for the Termination of Extracellular Endocannabinoid Signaling

Like typical neurotransmitters, endocannabinoids are translocated across the plasma membrane in order to cease their signaling at the extracellular cannabinoid receptors. However, the mechanism and proteins responsible for AEA and/or 2-AG transport remain elusive and hotly debated. While some researchers have proposed that these lipophilic endocannabinoids cross the cell plasma membrane via simple diffusion through the lipid bilayer (Glaser *et al.*, 2003;Glaser *et al.*, 2005;Kaczocha *et al.*, 2006), other data indicate that the uptake process is a protein-facilitated event (Hillard *et al.*, 1997;Hillard and Jarrahian, 2000;Rakhshan *et al.*, 2000;Beltramo and Piomelli, 2000). Numerous studies conducted in various cell types, both of neuronal and non-neuronal origin, have characterized AEA and 2-AG uptake as being temperature-dependent, saturable, and independent of energy in the form of ion gradients or adenosine

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triphosphate (ATP) hydrolysis (Rakhshan *et al.*, 2000;Maccarrone *et al.*, 2000;Hillard *et al.*, 1997;Hillard and Jarrahian, 2003;Hermann *et al.*, 2006;Deutsch *et al.*, 2001;Day *et al.*, 2001;Bisogno *et al.*, 2001;Beltramo and Piomelli, 2000). However, even among those in agreement with a protein-facilitated model for endocannabinoid uptake, there remains debate concerning the precise type of protein-facilitated event responsible.

Several different models have been proposed for endocannabinoid uptake that fit under the general heading of a protein-facilitated event: 1) transmembrane carrier (Hillard and Jarrahian, 2000;Beltramo *et al.*, 1997)), 2) intracellular sequestration (Hillard and Jarrahian, 2003;Hillard *et al.*, 2007), 3) passive diffusion driven by fatty acid amide hydrolase (FAAH) (Glaser *et al.*, 2003), and 4) carrier-mediated caveolae-related endocytosis (McFarland *et al.*, 2004;McFarland *et al.*, 2008;Rakhshan *et al.*, 2000). The majority of these models have been suggested as a result of experiments studying AEA transport only. Evidence exists though, which suggests that 2-AG and AEA are accumulated in cells via a common mechanism(s) (Beltramo and Piomelli, 2000). Both 2-AG and AEA uptake have been characterized as protein-facilitated events (Bisogno *et al.*, 2001;Beltramo and Piomelli, 2000). Additionally, 2-AG has been shown to inhibit AEA uptake in cells, indicating a competitive nature of the two endocannabinoids with regard to transport (Bisogno *et al.*, 2001;Beltramo and Piomelli, 2000).

*Transmembrane Carrier Protein*

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AEA diffusion across the lipid bilayer has been proposed to be facilitated by a membrane-localized protein carrier (Figure 1A) (Deutsch *et al.*, 2001;Hillard *et al.*, 1997;Hillard and Jarrahian, 2000;Ligresti *et al.*, 2004;Beltramo *et al.*, 1997). Much of the evidence for the existence of a membrane-localized endocannabinoid carrier protein stems from the observation that AEA transport in cells is bidirectional (Hillard *et al.*, 1997;Hillard and Jarrahian, 2000;Ligresti *et al.*, 2004;Maccarrone *et al.*, 2002). Studies conducted in both neuronal and non-neuronal cells demonstrate AEA efflux as well as uptake (Maccarrone *et al.*, 2002;Hillard *et al.*, 1997). In addition, experiments performed by Hillard and colleagues indicate that the elusive membrane-localized AEA carrier is capable of the trans-flux coupling effect, a phenomenon whereby in response to extracellular AEA, the membrane-localized carrier protein accumulates at the cell surface in the extracellular-facing direction (Hillard and Jarrahian, 2000).

*Intracellular Sequestration Model*

The intracellular sequestration of endocannabinoids by a fatty acid binding protein(s) is another proposed mechanism for endocannabinoid uptake suggested by Hillard and colleagues (Hillard and Jarrahian, 2003;Hillard *et al.*, 2007) (Figure 1B). Interestingly, this model simultaneously supports the proposition that AEA passively diffuses across the lipid bilayer and explains the characteristics of AEA uptake consistent with a protein-facilitated process. Following the unassisted translocation of AEA across the plasma membrane, the fatty acid-derived AEA may interact with fatty acid binding proteins (Hillard and Jarrahian, 2003). The intracellular sequestration of AEA by these binding

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proteins would remove AEA from the intracellular pool of "free" AEA, thus, promoting the inward concentration gradient and AEA uptake (Hillard and Jarrahian, 2003).

*FAAH-Driven Passive Diffusion*

FAAH-mediated hydrolysis of intracellular AEA does, to some extent, drive AEA uptake (Figure 1C). Our lab and others have shown that FAAH activity promotes AEA transport most likely by driving the concentration gradient along which AEA uptake occurs (Deutsch *et al.*, 2001;Day *et al.*, 2001;Cravatt *et al.*, 2001). Cells devoid of FAAH show diminished AEA accumulation as compared to those that basally express or over-express FAAH protein (Deutsch *et al.*, 2001;Day *et al.*, 2001). Additionally, recent evidence suggests that most "selective" AEA uptake inhibitors also inhibit FAAH activity (Dickason-Chesterfield *et al.*, 2006). This revelation subsequently begged the question as to whether or not a specific "AEA transport protein(s)" exists.

Intracellular enzymatic degradation is probably not solely responsible for the movement of endocannabinoids across the plasma membrane. The most compelling data arguing that FAAH alone is not responsible for endocannabinoid uptake comes from work with FAAH knockout mice where cells and tissues devoid of FAAH are still capable of accumulating AEA in a saturable and pharmacologically-manipulated manner (Ortega-Gutierrez *et al.*, 2004;Fegley *et al.*, 2004;Ligresti *et al.*, 2004). Similarly, Fowler and Ghafouri showed that 2-AG uptake is not prevented by pharmacological inhibition of 2-AG hydrolysis in all cell types, indicating possible cell-specific mechanisms for 2-AG uptake, but most

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importantly that hydrolysis is not the sole factor mediating transport (Fowler and Ghafouri, 2008).

Perhaps some of the most convincing evidence against FAAH being solely responsible for AEA uptake comes from the development of selective AEA uptake inhibitors. Ortar and colleagues announced their development of a series of tetrazole-based selective anandamide uptake inhibitors that do not inhibit FAAH or other metabolizing enzymes, thus, supporting the existence of a distinct protein target responsible for mediating endocannabinoid uptake (Ortar *et al.*, 2008). All of these data taken together suggest that, in addition to FAAH activity, a distinct protein-facilitated transport process is responsible for promoting the cellular accumulation of AEA.

*Carrier-Mediated Caveolae-Related Endocytosis*

Our lab has proposed that AEA uptake occurs via a protein carrier-mediated caveolae-related endocytic event (Figure 1D) (Rakhshan *et al.*, 2000;McFarland *et al.*, 2004;McFarland *et al.*, 2008). We demonstrated that inhibition of caveolae-related endocytosis or prevention of caveolae formation both led to a significant decrease in cellular AEA accumulation, thus, implicating a role for these membrane microdomains in the AEA uptake process (McFarland *et al.*, 2004;McFarland *et al.*, 2008). We propose that extracellular AEA binds a carrier protein located within caveolae, and that subsequently, caveolae-derived vesicle formation and endocytosis of the membrane-packaged endocannabinoid is induced (McFarland *et al.*, 2004;McFarland *et al.*, 2008;McFarland and Barker, 2004). The subsequent delivery of internalized AEA to FAAH may be a critical

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step in freeing up the carrier protein for additional AEA transport events. As described, carrier-mediated endocytosis could be used to reconcile most of the other models for endocannabinoid uptake discussed above, including: the FAAH-mediated maintenance of the AEA concentration gradient; the existence of a membrane-localized AEA binding protein; and the possible sequestration of intracellular AEA.

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## Catabolic Degradation as a Mechanism for Terminating Endocannabinoid Signaling

Following cellular uptake, AEA and 2-AG are subject to metabolism by the serine hydrolases fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Additionally, AEA and 2-AG have been shown to undergo oxidation by cyclo-oxygenase-2 (COX-2) and the 12- and 15-lipoxygenases (12-LOX and 15-LOX) (Di Marzo, 2006).

Catalytic degradation/modification of AEA and 2-AG not only serves as a mechanism for the augmentation of cellular uptake and cessation of extracellular signaling as mentioned above, but also regulates the intracellular signaling events of these two endocannabinoids. Below, we will briefly review the roles of the aforementioned enzymes in AEA and 2-AG metabolism.

### *Endocannabinoid Hydrolysis: FAAH1, FAAH2, and MAGL*

*FAAH1 and FAAH2* FAAH-mediated hydrolysis of AEA yields arachidonic acid and ethanolamine (Figure 2A) (Deutsch and Chin, 1993). Currently, two FAAH isoforms (FAAH1 and FAAH2) have been identified (Cravatt *et al.*, 1996;Wei *et*

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*al.*, 2006). The intracellularly-localized FAAH1 and FAAH2 are both members of the amidase signature protein family and share approximately 20 percent sequence identity (McKinney and Cravatt, 2005;Wei *et al.*, 2006;Giang and Cravatt, 1997;McFarland *et al.*, 2004). Both isoforms are integral membrane proteins, owing to a single transmembrane domain on their respective N-termini, although their orientation in the membrane differs (Cravatt *et al.*, 1996;Wei *et al.*, 2006). FAAH1 has been proposed to contain a cytoplasmic-facing C-terminus, whereas the C-terminus of FAAH2 faces the lumen (Wei *et al.*, 2006). The two FAAH isoforms also vary in their expression patterns (Wei *et al.*, 2006). FAAH1 has been cloned from several different species, including mice, rats, and humans and is preferentially expressed in the brain, testis, and small intestine (McKinney and Cravatt, 2005;Wei *et al.*, 2006). FAAH2 is not expressed in rodents and is the predominant isoform found in cardiac tissue (Wei *et al.*, 2006). Also, FAAH1 has been reported to have greater activity with regard to fatty acid ethanolamides such as AEA (Wei *et al.*, 2006).

*MAGL* Although some reports suggest that FAAH may also play a role in 2-AG degradation (Di Marzo, 2006), the major enzyme responsible for 2-AG metabolism appears to be the serine hydrolase MAGL (Dinh *et al.*, 2002). MAGL has no sequence similarity with any member of the amidase signature protein family, including either FAAH isoform, or any other mammalian protein (Saario and Laitinen, 2007). However, MAGL does contain the α/β-hydrolase fold common to many lipases (Saario and Laitinen, 2007). As a proposed serine hydrolase, MAGL is capable of hydrolyzing both medium- and long-chain fatty

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acids (Saario and Laitinen, 2007). MAGL-mediated hydrolysis of 2-AG yields arachidonic acid and glycerol (Figure 2B) (Karlsson *et al.*, 1997). Overexpression and siRNA-mediated knockdown of MAGL results in increased and decreased 2-AG inactivation, respectively (Dinh *et al.*, 2004;Dinh *et al.*, 2002). MAGL protein is expressed in a variety of human, rat, and mouse tissues (Saario and Laitinen, 2007;Long *et al.*, 2009).

*Endocannabinoid Oxidation: COX-2 and the 12- and 15-Lipoxygenases*

AEA and 2-AG are not only subject to hydrolysis, but because of their structure can also be metabolized by several of the same enzymes that are responsible for arachidonic acid oxidation, including COX-2 and the 12- and 15-lipoxygenases (Di Marzo, 2006).

*COX-2* COX-2 is responsible for catalyzing the oxidation of AEA and 2-AG into various prostaglandin-ethanolamides (PG-EAs or prostamides) and prostaglandin-glycerol esthers (PG-GEs), respectively (Figure 3A and B) (Woodward *et al.*, 2008). Until recently, whether or not such metabolites existed *in vivo* was unknown. However, Hu and colleagues have provided evidence to suggest that, indeed, at least some such *in vivo* reactions do occur (Shu-Jung Hu *et al.*, 2008). Interestingly, the endocannabinoid-derived prostaglandins have unique pharmacological profiles that appear to involve as-of-yet unidentified receptors (Shu-Jung Hu *et al.*, 2008;Woodward *et al.*, 2008;Di Marzo, 2006).

*Lipoxygenases* AEA and 2-AG have also been identified as substrates for both 12-LOX and 15-LOX in intact cells . Oxidative metabolism of AEA by 12-LOX and 15-LOX results in the formation of 12- and 15-

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hydroperoxyeicosatetraenoylethanolamide (12-HETE-EA and 15-HETE-EA), respectively (Figure 4A) (Edgemond *et al.*, 1998;Veldhuis *et al.*, 2003). Likewise, 12-LOX- and 15-LOX-mediated oxidation of 2-AG results in the formation of 12- and 15-hydroperoxyeicosatetraenoic acid glycerol ester (12-HETE-GE and 15-HETE-GE), respectively (Figure 4B) (Kozak *et al.*, 2002;Moody *et al.*, 2001). Unlike the endocannabinoid-derived prostaglandins, the lipoxygenase derivatives of AEA and 2-AG appear to mediate their biological activities via established receptors, including the cannabinoid receptors, PPAR-α, and TRPV1 (Di Marzo, 2006;Edgemond *et al.*, 1998;Kozak *et al.*, 2002;Craib *et al.*, 2001).

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## Pharmacological Manipulation of Endocannabinoid Uptake and Metabolism

The cannabinoid system is currently an active research area due to the many physiological and pathophysiological implications associated with AEA and 2-AG signaling such as appetite (Kirkham and Tucci, 2006), pain (Hohmann and Suplita, 2006), anxiety (Witkin *et al.*, 2005), fertility (Wang *et al.*, 2006), neurodegeneration (Battista *et al.*, 2006), the immune response (Ashton, 2007), and cardiac health (Ashton and Smith, 2007). Pharmacological manipulation of endogenous AEA and 2-AG levels is one way to selectively regulate their associated signaling events for therapeutic purposes. Thus, the proteins involved in endocannabinoid uptake and metabolism, the events responsible for termination of endocannabinoid signaling, are attractive targets for pharmacological exploitation aimed at modulating AEA and 2-AG signaling.

### *The Search for Selective Endocannabinoid Reuptake Inhibitors (SERIs)*

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Unfortunately, the combination of the elusiveness of the protein(s) responsible for AEA and/or 2-AG uptake along with the unresolved relationship that exists between endocannabinoid uptake and FAAH/MAGL activity has hindered the development of SERIs. In fact, one 2006 study showed that nearly all "selective" AEA uptake inhibitors also block FAAH activity to one extent or another (Dickason-Chesterfield *et al.*, 2006).

Yet, recent developments indicate that the identities of the endocannabinoid transporter(s) may soon be determined. Moore and colleagues announced their development of the potent tetrazole-based specific AEA uptake inhibitor LY2318912 (Moore *et al.*, 2005). This compound appears to bind a protein target distinct from FAAH and does not cross the cell membrane, supporting the hypothesis of a plasma membrane localized or associated AEA carrier (Moore *et al.*, 2005). Additionally, several new molecules designed to isolate and identify the putative transporter protein(s) have recently been developed, including several photo-affinity radioligands as well as a biotin-tagged AEA (Balas *et al.*, 2005;Balas *et al.*, 2006;Fezza *et al.*, 2008;Moore *et al.*, 2005;Moriello *et al.*, 2006).

The increased specificity for endocannabinoid uptake inhibition exhibited by some of the tetrazole-based compounds may prove to be useful not only in the identification of the elusive protein(s) involved in endocannabinoid transport, but also in dissecting the role of endocannabinoid signaling in certain physiological and behavioral phenomena. Trezza and Vanderschuren suggest that the results of *in vivo* studies utilizing endocannabinoid reuptake inhibitors

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may be affected by the specificity of the inhibitor used (Trezza and Vanderschuren, 2009). They found that the non-specific uptake inhibitor AM404 and the more-specific uptake inhibitor VDM11 had contradicting effects on the social play of rats possibly due to off-target effects elicited by AM404 that are unassociated with CB1, CB2, or TRPV1 receptors (Trezza and Vanderschuren, 2009). Thus, compounds that specifically inhibit endocannabinoid uptake will better elucidate the true behavioral and physiological consequences of augmented endocannabinoid signaling.

Although many compounds initially thought to be specific for the endocannabinoid transporter have been shown to also inhibit FAAH and/or MAGL, these non-specific AEA uptake inhibitors do have utility in endocannabinoid research. For instance, *in vivo* studies using AM404 have implicated the endocannabinoid system in the neuropathic and inflammatory pain pathways (La Rana *et al.*, 2008;Mitchell *et al.*, 2007) as well as in the mediation of antidepressant-like effects (Adamczyk *et al.*, 2008). AM404 has also been shown to reduce ethanol administration in rats, suggesting utility for the compound in the treatment of alcoholism, although the exact signaling pathway responsible for this effect is unknown (Cippitelli *et al.*, 2007).

*Inhibitors of AEA and 2-AG Hydrolysis*

In instances where increased AEA or 2-AG signaling may have therapeutic benefit such as chronic pain or anxiety, inhibition of AEA and 2-AG enzyme-mediated hydrolysis may be desirable. Specifically inhibiting FAAH could increase AEA signaling in two ways: 1) by preventing AEA hydrolysis and,

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2) by decreasing the rate of AEA uptake into cells by interfering with the inward concentration gradient perpetuated by intracellular AEA hydrolysis.

In addition, the metabolites of AEA and 2-AG hydrolysis may themselves play roles in disease. For instance, 2-AG metabolites have been implicated as stimulatory factors in the pathogenesis of prostate cancer (Endsley *et al.*, 2007). Endsley and colleagues observed that in the androgen-independent prostate carcinoma (PC-3) cells, exogenous application of 2-AG increased production of arachidonic acid, which is then oxidized by 12-lipoxygenase. The resulting oxidized product, 12-HETE-GE, stimulates prostate cell invasion (Endsley *et al*., 2007). The authors propose that inhibition of 2-AG hydrolysis in such instances may prove to have therapeutic potential (Endsley *et al.*, 2007).

Over the years, a significant number of FAAH inhibitors have been developed (for a review, see Seierstad and Breitenbucher, 2008). In addition to the development of FAAH inhibitors, some compounds currently on the market, including several NSAIDS, have been shown to inhibit FAAH activity (Fowler *et al.*, 2001;Seierstad and Breitenbucher, 2008). However, the development of MAGL inhibitors has lagged historically. Evidence suggests that boronic acids potently inhibit FAAH and may serve as good starting compounds for the development of better MAGL inhibitors (Minkkila *et al.*, 2008). Recently, Long and colleagues announced their development of a selective MAGL inhibitor JZL184 that produces antinociceptive effects, hypomotility, and hypothermia in mice (Long *et al.*, 2009). This advancement offers many possibilities not only for therapeutic development of MAGL inhibitors, but also with regard to research

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aimed at dissecting the overlapping and distinct effects of AEA and 2-AG signaling.

*Endocannabinoid-derived Oxidative Metabolites as Pharmacological Targets*

There have been many pathophysiological implications for the endocannabinoid-derived oxidative metabolites produced by COX-2 and the 12- and 15-LOXs. For example, data exist suggesting that COX-2-mediated oxidation of endocannabinoids plays an important regulatory role in hippocampal neuronal transmission and synaptic plasticity (Yang *et al.*, 2008;Sang *et al.*, 2006). Additionally, as briefly mentioned earlier, 12-LOX-generated oxidative metabolites of AEA may be agonists for TRPV1, a key channel in pain modulation (Craib *et al.*, 2001). These are just two examples of cell signaling events potentially mediated by the oxidative metabolites of AEA and 2-AG. Many questions still remain regarding the signaling fates of these and other endocannabinoid metabolites.

This mini-review has examined the various ways in which the endocannabinoids AEA and 2-AG may be inactivated. Endocannabinoid inactivation can be pharmacologically modulated at the level of cellular accumulation or intracellular metabolism. Metabolism occurs predominantly via the hydrolytic enzymes FAAH and MAGL or the oxidative enzymes COX-2, 12-LOX, and 15-LOX. The molecular process responsible for the intracellular accumulation of AEA and 2-AG has yet to be fully elucidated, but the majority of data to date suggest that endocannabinoid uptake is protein-facilitated. Much still remains to be learned regarding these events, including the identity of the

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putative endocannabinoid transporter(s) as well as identification of the signaling events mediated by the metabolic derivatives of AEA and 2-AG.

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*Legends for Figures*

Figure 1. Various proposed models for endocannabinoid transport. Although the majority of these models were developed based on data from AEA uptake studies, there is some evidence to suggest that AEA and 2-AG uptake occur via a common mechanism. *A)* A transmembrane carrier protein assists in the translocation of endocannabinoids across the plasma membrane; *B)* Endocannabinoids passively diffuse across the plasma membrane along a catabolism-driven concentration gradient, but are sequestered in an intracellular compartment or by binding to an intracellular binding protein prior to metabolism; *C)* Endocannabinoids passively diffuse across the plasma membrane along a concentration gradient that is driven by their rapid metabolism; *D)* Endocannabinoids are transported into cells via a protein carrier-mediated caveolae-related endocytic event.

Figure 2. Hydrolysis of the endocannabinoids AEA and 2-AG. *A)* FAAH catalyzes the hydrolysis of AEA into arachidonic acid and ethanolamine. *B)* MAGL catalyzes the hydrolysis of 2-AG into arachidonic acid and glycerol.

Figure 3. The major metabolites generated via COX-2-mediated oxidation of the endocannabinoids *A)* AEA and *B)* 2-AG. Prostaglandin E2 ethanolamide, $PGE_2$-EA; prostaglandin E2 glycerol esther, $PGE_2$-GE.

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Figure 4. The major metabolites generated via oxidation of *A)* AEA and *B)* 2-AG by the 12- and 15-LOXs. Hydroperoxyeicosatetraenoylethanolamide, HETE-EA; hydroperoxyeicosatetraenoic acid glycerol ester, HETE-GE.

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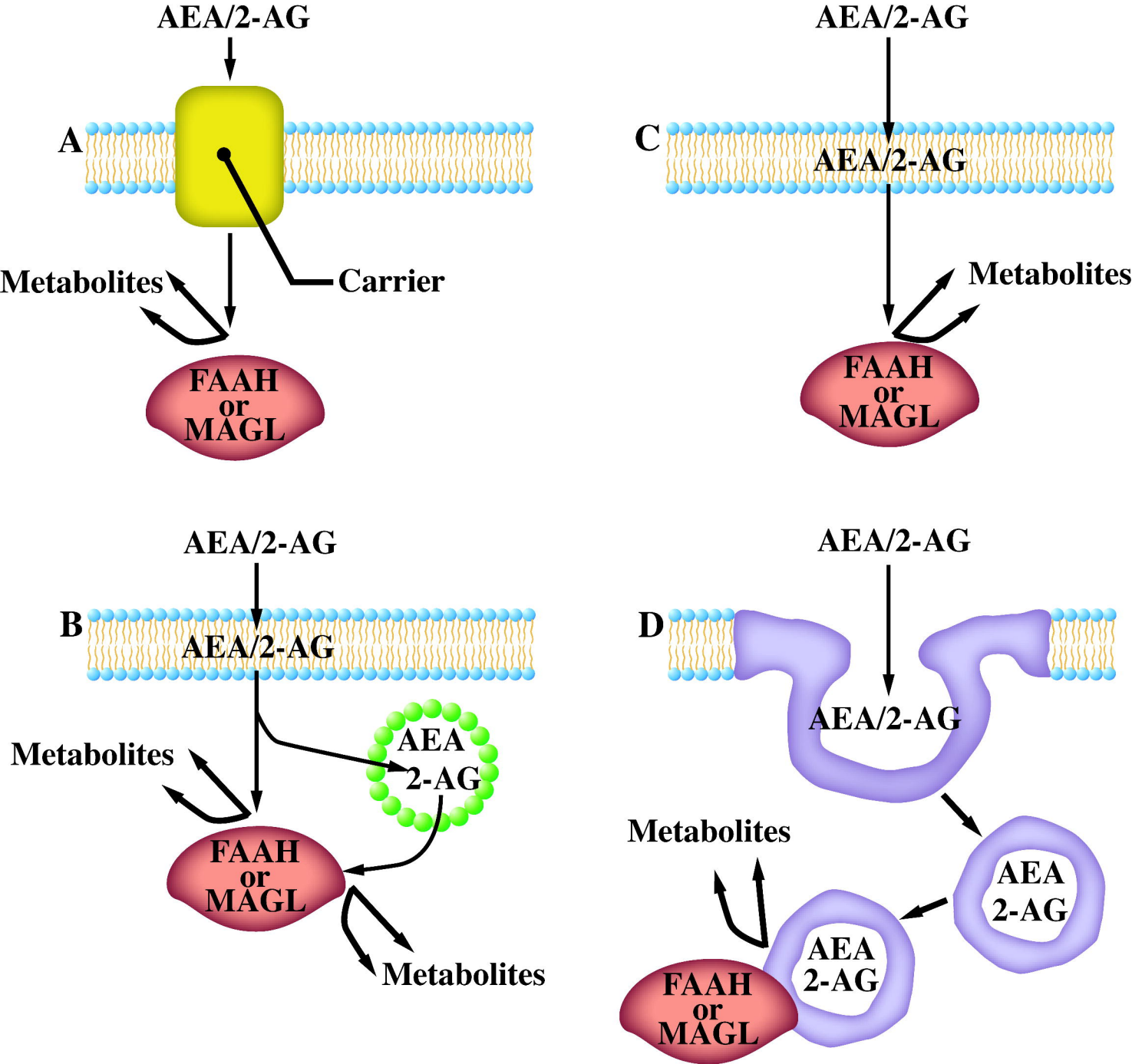
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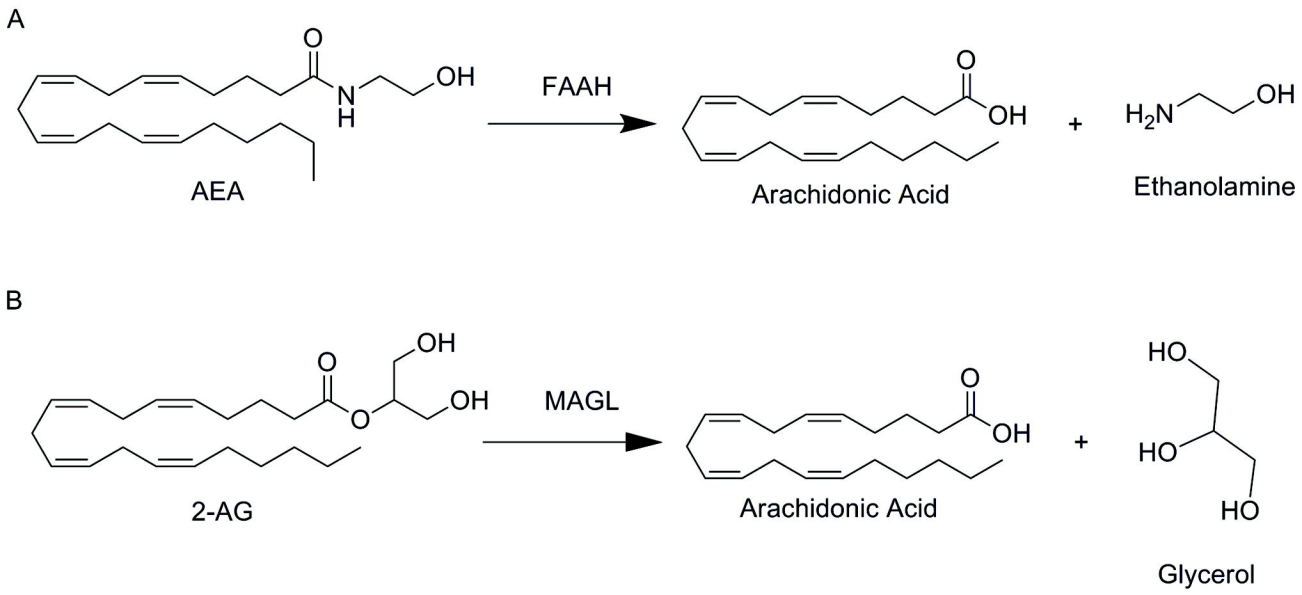
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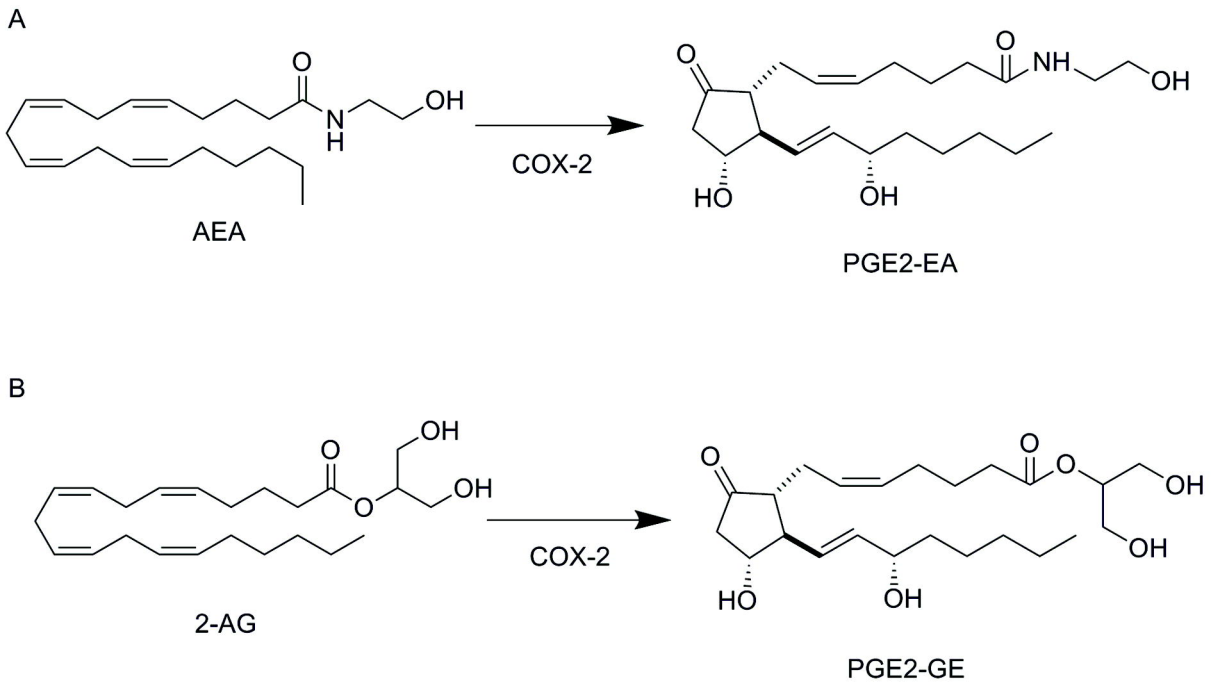
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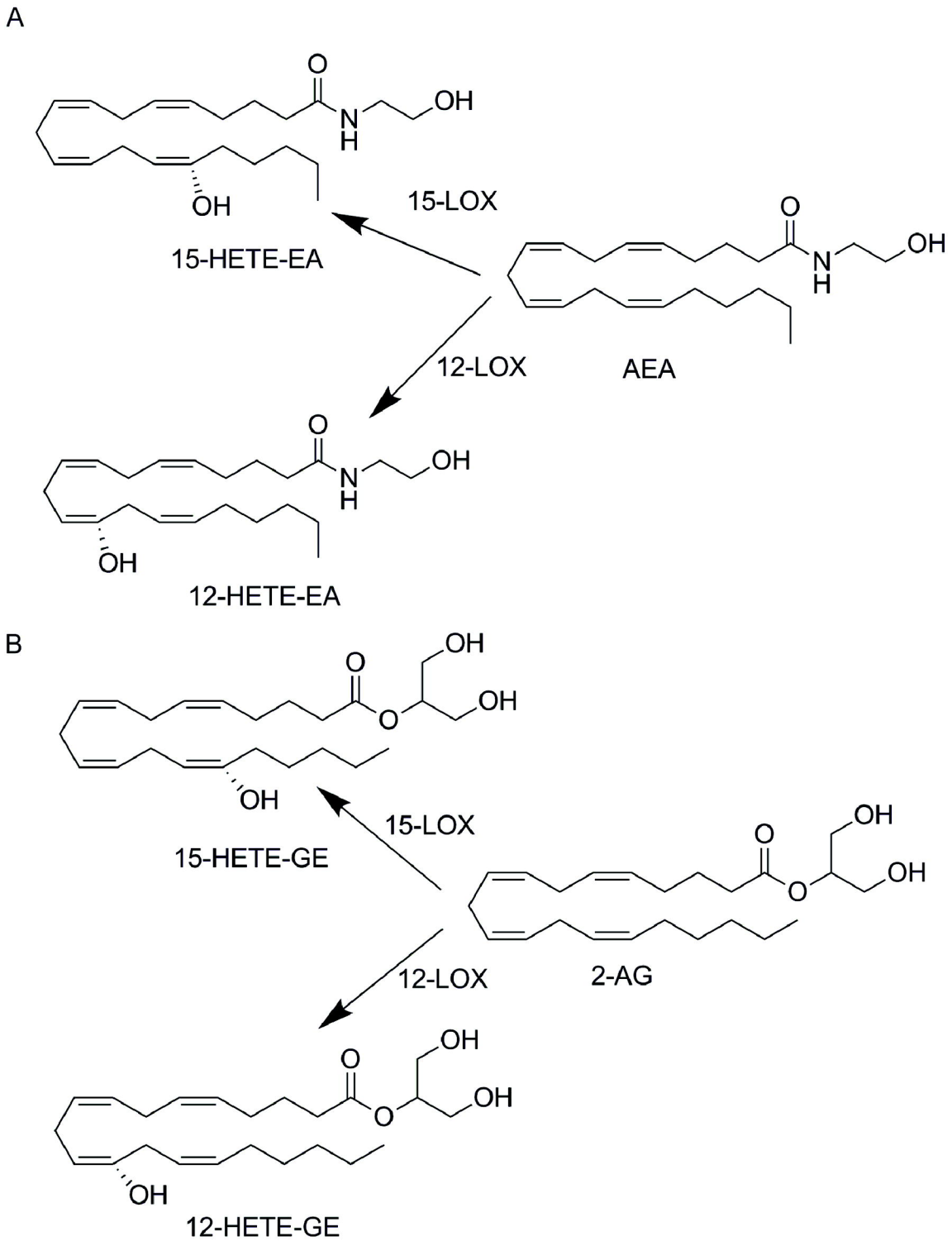
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