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Emerging Class of Omega-3 Fatty Acid Endocannabinoids & Their Derivatives

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Abstract

Cannabinoid receptor activation is involved in homeostatic regulation of the body. These receptors are activated by cannabinoids, that include the active constituents of *Cannabis sativa* as well as endocannabinoids (eCBs). The eCBs are endogenously synthesized from the omega-6 and omega-3 polyunsaturated fatty acids (PUFAs). The consumption of omega-3 fatty acids shifts the balance towards a higher proportion of omega-3 eCBs, whose physiological functions warrants further investigation. Herein, we review the discovery of omega-3 fatty acid derived eCBs that are generated from long chain omega-3 PUFAs - docosahexaenoyl ethanolamide (DHA-EA or synaptamide), docosahexanoyl-glycerol (DHG), eicosapentaenoyl ethanolamide (EPA-EA), eicosapentanoylglycerol (EPG). Furthermore, we outline the lesser known omega-3 eCB-like molecules that arise from the conjugation of the omega-3 fatty acids with neurotransmitters serotonin and dopamine - DHA-serotonin (DHA-5HT), EPA-serotonin (EPA-5HT), DHA-dopamine (DHA-DA) and EPA-dopamine (EPA-DA). Additionally, we describe the role of these omega-3 eCBs and their derivatives in different disease states such as pain, inflammation and cancer. Moreover, we detail the formation and potential physiological roles of the oxidative metabolites that arise from the metabolism of omega-3 eCBs by eicosanoid synthesizing enzymes - cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 epoxygenase (CYP450). In summary, we outline the novel findings regarding a growing class of signaling molecules, omega-3 eCBs, that can control the physiological and pathophysiological processes in the body.

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Authors' Contributions

J.E.W. and J.S.K. contributed equally to the manuscript. J. E. W and J. S. K performed literature review, wrote the review and made figures and tables. A.D. performed literature review and edited the review.

Conflict of Interest Statement

The authors declare no competing financial interest.

Keywords

Endocannabinoids; omega-3 fatty acids; inflammation; cancer; metabolism

Introduction

Cannabis sativa is used for both medical and recreational use (1, 2). In the early 1950s, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the primary psychoactive molecule, was isolated from *Cannabis sativa* (3). This led to a strong interest in the synthesis of cannabinoid (CB) analogs to understand the structure-activity relationship with putative CB receptors (4–8). Eventually, two G-protein coupled receptors (GPCRs), cannabinoid receptor 1 and 2 (CB₁ and CB₂), were discovered (9, 10). While CB₁ is highly expressed in the central nervous system (CNS), CB₂ is present primarily in immune and peripheral cells. Since Δ^9 -THC and other CBs that bind to CB₁ and CB₂ are lipophilic, there was a search for endogenous lipids that interacted with these receptors.

In the 1990s, the first endogenous cannabinoids (eCBs) that were shown to activate CB₁ were anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (11–13). AEA and 2-AG are synthesized from omega-6 polyunsaturated fatty acid (PUFA), arachidonic acid (AA), and are hydrolyzed by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Later studies identified other eCB-metabolizing enzymes, such as FAAH 1 and 2 for AEA; MAGL, α , β -hydrolase domain-containing 6 and 12 (ABDH6 and ABDH12) for 2-AG. Both AEA and 2-AG are also metabolized by eicosanoid synthesizing enzymes such as lipoxygenases (LOX), cyclooxygenase 2 (COX-2) and cytochrome P450 epoxygenases (CYP450) to form new bioactive molecules (14, 15). Together, the interactions between the CB receptors, eCB ligands, as well as their biosynthetic and degradative enzymes, constitute the eCB system (ECS) and are involved in maintaining homeostasis in the biological system (16).

Broadly, the eCBs are members of a large group of structurally related fatty acid derived signaling molecules. It is important to note that eCBs are traditionally defined as ligands with significant affinity and agonism to CB₁ and CB₂ according to the NC-IUPHAR committee (17). This definition resulted in the discovery and synthesis of several other eCB and “eCB-like” analogues (18–21). Thus, many of these fatty acid derived molecules are eCB-like ligands that have weak affinity to CB receptors and cannot be considered as classical eCBs. Additionally, as the definition of the ECS system is expanding, it is currently known that CBs and other eCB-like compounds, such as N-arachidonoylglycine (NA-Gly), can target more receptors, such as TRPV1, GPR18, GPR119 and GPR55 (22–26).

With the increasing focus towards the consumption of omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), there has been a growing interest in the discovery of omega-3 fatty acid derived eCB or eCB-like molecules with novel bioactivity (27). Herein, we review the discovery and physiological role of omega-3 eCBs that are derived from DHA and EPA (Figure 1A). We illustrate that the derivatization of carboxylic acid end of the omega-3 fatty acid with ethanolamide, glycerol or other groups, such as neurotransmitters, leads to substantial changes in their biological activity and receptor

interactions. We also review the discovery, mechanism and activity of the following omega-3 eCBs- DHA-ethanolamide (DHA-EA), 2-docosahexaenoyl-glycerol (2-DHG) and eCB-like-DHA-serotonin (DHA-5HT), DHA-dopamine (DHA-DA) and the EPA analogs.

Additionally, we also evaluate the formation and potential physiological roles of their oxidative metabolites, which arise from the metabolism of omega-3 eCBs by eicosanoid synthesizing enzymes.

1. Omega-3 fatty acid DHA ethanolamide (DHA-EA or DHEA)

In order to study the structure-activity relationship of eCB-like analogues to CB₁, an omega-3 derivative of DHA, N-docosahexaenylethanolamide (DHA-EA or DHEA), was synthesized. In comparison to AEA, DHA-EA was shown to be 8- to 22.5-fold less potent to CB₁ receptor (Figure 1B & Table 1) (12, 28). Later, DHA-EA was found endogenously in brain and retina (29, 30). When piglets were supplemented with a DHA diet, a several fold increase in DHA-EA levels were measured (Table ST1) (30). Additionally, dietary consumption of fish oil led to higher levels of DHA-EA in rodent brain, jejunum, liver and adipose tissue (Table ST1) (31–34). DHA-EA was also measured under inflammatory conditions in rat cerebrospinal fluid and the spinal cord tissue (Table ST1) (35). Most recently, DHA-EA has been detected in human milk (Table ST1) (36). As DHA-EA is identified in the body, further investigation of its physiological and pharmacological roles are necessitated.

1.1 Role of DHA-EA in the Central Nervous System (CNS)

Since DHA-EA is detected in the brain, its role in synaptogenesis (formation of synapses between neurons) were investigated (37). Previously, DHA-EA production in neurons correlated with enhanced glutamatergic synaptic activity (38, 39). This activity is required for the synaptogenesis process; hence, DHA-EA was named “synaptamide” (40). In dose-dependent studies, DHA-EA significantly induced synaptic activity at 0.1 μM and 1 μM. Additionally, DHA-EA treatment increased hippocampal neurite growth 10-fold more than that of DHA following three days of supplementation. Similar to AEA, DHA-EA is hydrolyzed to DHA by FAAH. Interestingly, in the presence of a FAAH inhibitor, the synaptogenic properties of DHA were potentiated and DHA-EA levels increased (39, 40). Collectively, results from these studies helped in establishing the dual significance of the combined amide moiety and omega-3 scaffold in promoting synaptogenesis (Table 2) (41).

Given that eCBs elicit their activities through receptor activation, it was hypothesized that DHA-EA promoted synaptogenesis by acting as an endogenous ligand of GPR110. In the study, use of GPR110 KO mice abolished DHA-EA bioactivity by significantly reducing synaptic protein expression and synapse number in developing brains. DHA-EA also induced neurogenic differentiation and neurite outgrowth, which were also dependent upon GPR110 activation. Hence, the DHA-EA/GPR110 signaling is hypothesized to be an essential mechanism for active neurogenesis and synaptogenesis, proving vital for the development of brain function (42).

The role of DHA-EA in neural development may further be supported by the detection of DHA-EA in murine embryonic stem cells and neural stem cells (NSCs) (43, 44). In NSCs,

DHA-EA treatment induced neuronal differentiation for seven days. The maximum effect for DHA-EA was measured at 10 nM alone and at 5 nM in the presence of a FAAH inhibition. At nanomolar concentrations, DHA-EA was 50 to 100-fold more effective, than DHA, at increasing expression of neuron-specific markers, microtubule-associated protein 2 (MAP2) and class III beta-tubulin (Tuj-1). Interestingly, DHA-EA also increased phosphorylation of protein kinase A (PKA) substrates, which have been demonstrated to mediate the CREB signaling pathway (44). For instance, ethanol reduced levels of cAMP and hindered the ability of NSCs to differentiate, yet, DHA-EA treatment reversed these ethanol-induced effects. Additionally, at concentrations between 1 and 10 nM, DHA-EA significantly increased the cellular cAMP production and upregulated expression of adenylyl cyclase's, AC7 and AC8, which are important regulators of cAMP levels. Notably, in the presence of 25 mM ethanol, 1 to 5 nM of DHA-EA completely reversed cAMP reduction (45).

Furthermore, DHA-EA has shown to promote axon development by negatively regulating sonic hedgehog (Shh) signaling in cortical neurons (46). Shh signaling is activated when Shh binds to patched-1 receptor that leads to transactivation of a smoothened GPCR (SMO). In the presence of SMO, 1 μ M DHA increased levels of DHA-EA and caused a 28% increase in cortical neuron growth. Additionally, at 100 nM, DHA-EA prevented SMO-dependent transcription of GLI family zinc finger and blocked signaling induced by Shh. Intriguingly, when SMO was overexpressed in cortical cells, these cells failed to significantly decrease axonal growth caused by DHA-EA. Yet, in the presence of adenylyl cyclase inhibition, DHA-EA axonal growth was decreased, highlighting the demand for cAMP to drive this process. Collectively, these studies showed that DHA-EA enhances synapto- and neurogenesis several folds more than DHA (44–46).

1.2 Role of DHA-EA in Inflammation

DHA-EA has been shown to be anti-inflammatory in several different inflammation models. For instance, the production and anti-inflammatory properties of DHA-EA were first demonstrated in 3T3-L1 adipocytes (Table 2). After 24 hours, 10 to 50 μ M of DHA supplementation increased the conversion of DHA to DHA-EA by 2- to 7-fold higher compared to controls. To investigate the properties of DHA-EA in adipocytes, a pro-inflammatory response was evoked using lipopolysaccharide (LPS), a component from gram-negative bacteria commonly used to study inflammatory processes. DHA-EA supplementation at 1 nM to 10 μ M reduced production of pro-inflammatory cytokines, such as IL-6 and monocyte chemotactic protein-1 (MCP-1), in differentiated adipocytes. Notably, both markers were reduced by 50% in the presence of 10 nM DHA-EA, compared to DHA, which had no effect at this concentration (47). Furthermore, receptor inhibition studies demonstrated that the reduction of pro-inflammatory markers were partially mediated by PPAR γ and CB $_2$. These studies confirmed that DHA-EA in adipocytes exhibit anti-inflammatory properties that are mediated through both CB- and non-CB receptors mediated pathways (47).

The effects of DHA-EA were further investigated in macrophages. At 10 μ M, DHA-EA significantly inhibited pro-inflammatory nitric oxide (NO) and MCP-1 production in LPS-

stimulated RAW264.7 and peritoneal macrophages (Table 2) (48). To elucidate the potential role of toll-like receptor (TLR) signaling in the anti-inflammatory effects caused by DHA-EA, the involvement of the TLR3/TLR4 myeloid differentiation primary response 88 pathway was examined. Interestingly, DHA-EA treatment in LPS and poly-IC induced macrophages led to significant reduction in the level of NO. However, DHA-EA did not inhibit NF- κ B or IFN- β activation that is associated with this TLR3/TLR4 pathway. Additionally, pharmacological inhibition studies determined that CB₁, CB₂, and PPAR γ minimally contributed to NO reduction by DHA-EA. Further suggesting that reduction in NO production is mediated through an unknown or alternate pathway. Currently, it is hypothesized that DHA-EA may indirectly reduce the pro-inflammatory response by reducing AA-derived COX eicosanoids or it is converted further by eicosanoid synthesizing enzymes to form anti-inflammatory lipid metabolites (49).

1.3. Role of DHA-EA in Cancer

The bioactivity of omega-3 eCBs have also been demonstrated in cancer models. In this study, DHA-EA significantly induced the cell death of prostate cancer cells sensitive to androgen (LNCaP) and insensitive to androgen (PC3). Moreover, expression of CB₁ and CB₂ in PC3 and LNCaP were determined and radioligand binding studies confirmed DHA-EA as an agonist of both the CB receptors. Additionally, degradation of DHA-EA by FAAH was measured and the inhibition of this enzyme in LNCaP cells led to a potent increase in its anti-proliferative properties (18). In other cancer cell types, such as breast cancer cells, DHA-EA mediated anti-proliferation effects through the PPAR γ activation pathway (50).

1.4. Role of DHA-EA in Exercise and Pain

In recent years, eCBs have been evaluated for their potential role in exercise and pain. Exercise-induced hypoalgesia (EIH) is characterized by elevations in pain tolerance which has been attributed to the involvement of opioid and non-opioid systems. Previously, activation of CBs receptors and EIH have shown to lower nociceptor activity, enhance exercise-induced analgesia and release endogenous opioid-like molecules to modulate and reduce pain (51–53). Thus, it has been suggested that EIH may be reduced via elevated levels of eCBs after exercising to reduce pain sensitivity. In fact, DHA-EA levels become significantly increased in human serum after exercise (54). Additionally, exogenous treatment of DHA-EA to muscle tissue increased glucose-uptake and expression of CB₁, CB₂, and NAPE-PLD, a key enzyme involved in the synthesis of AEA (Table 2) (55).

DHA-EA bioactivity was also demonstrated in humans undergoing a high omega-3 and low omega-6 fatty acid diet intervention. This diet was shown to reduce headache frequency, headache severity, and psychological distress. Adults undergoing a high omega-3 and low omega-6 fatty acid diet intervention had significantly higher levels of DHA-EA in circulation, but lower levels of 2-AG in blood plasma (Table ST1). Notably, DHA-EA also caused an increase of anti-nociceptive omega-3 metabolites, such as 18-HEPE and 17-HDHA. Individuals on a high omega-3 diet reduced medication use for pain by 37%, while these results were not paralleled in a high omega-6 diet. Collectively, this evidence demonstrates that omega-3 dietary interventions increase the production of DHA-EA.

Likewise, when DHA-EA increases in circulation, a downstream increase in other eCBs and DHA metabolites that may aid in reducing pain activities (56).

1.5. Biosynthesis of DHA-EA

DHA-EA was first isolated from bovine retina. A potential precursor, N-docosahexanoyl-phosphatidylethanolamine (NDHPE), was identified (29). There are three hypothesized models for DHA-EA endogenous biosynthesis. Similar to AEA synthesis, one mechanism involves a phospholipase D2 enzyme that cleaves NDHPE from the phospholipid backbone to form free DHA-EA. Secondly, it is possible that α/β -hydrolase 4 can be used to create an intermediate susceptible to hydrolysis of the NDHPE such that free DHA-EA is released. Thirdly, direct condensation of DHA and ethanolamine to form DHA-EA can occur by FAAH, though this reversed hydrolysis would likely occur at significantly higher concentrations. Collectively, more studies are needed to elucidate the synthesis of DHA-EA and confirm which mode(s) of synthesis occurs predominantly *in vivo* (29, 57).

1.6. Metabolism of DHA-EA and Role of Metabolites

The eCBs are further metabolized by three eicosanoid synthesizing enzymes – COX, LOX and CYP450 – to form eCB metabolites. Studies have extensively investigated the generation of AEA and 2-AG oxy metabolites that have been produced through these enzymes (15). In recent years, DHA-EA was proposed to be a substrate for the LOX and CYP450 enzymes (Figure 2). The first reported metabolism of DHA-EA was through the LOX pathway. The primary DHA-EA metabolites were identified in mouse brain, human hemoglobin and polymorphonuclear (PMN) leukocytes (Figure 2A). During inflammation, PMN leukocytes migrate to the originating sites of inflammation, a process known as chemotaxis. In contrast, chemotaxis can be reduced in the presence of anti-inflammatory molecules, such as resolvins (58). Among the LOX products formed, 10,17-diHDHA-EA and 15-HEDPEA reduced the migration of PMN chemotaxis up to 10 nM and decreased platelet-leukocyte aggregation formation by ~30–40% in human whole blood at 10 and 100 pM (Table 2). In addition, 10,17-diDHA-EA and 15-HEDPEA demonstrated CB₂ receptor agonist activity with EC₅₀ values at 3.9 nM and 1 nM, respectively (Table 1) (20).

Recently, our group demonstrated that the CYP450 pathway plays a role in the metabolism of DHA-EA (Figure 2B). We have developed a targeted liquid chromatography tandem mass spectroscopy (LC-MS/MS) method to quantitatively measure and detect DHA-EA epoxide metabolites in rat tissues. Additionally, when rat brain homogenates were incubated with DHA-EA, six DHA-EA epoxide regioisomers called epoxydocosapentaenoic-ethanolamides (EDP-EAs) were produced. As proof of concept, one brain CYP450 isozyme, CYP2J2, was shown to be able to metabolize DHA-EA to form the corresponding epoxides. Furthermore, one regioisomer, 19,20-EDP-EA, was predominately produced by CYP450s in rat brain. This metabolite significantly reduced pro-inflammatory markers, IL-6 and NO, while inducing the expression of anti-inflammatory marker, IL-10, in microglial cells. Additionally, 19,20-EDP-EA mediated platelet aggregation, vasodilation, angiogenesis, while activating CB₁ and CB₂ receptors with EC₅₀ values of 108 nM and 280 nM, respectively (Table 1 and 2) (19). These studies suggest that omega-3 eCB metabolites may serve as potential therapeutic targets in neuroinflammation and cerebrovascular diseases.

2. Omega-3 fatty acid EPA ethanolamide (EPA-EA or EPEA)

When AEA was discovered to bind and activate CB₁, researchers synthesized AEA analogs to investigate which structural requirements were essential for binding to CB₁. One of the compounds synthesized was eicosapentanoyl ethanolamide (EPA-EA or EPEA) (Figure 1B) (59). Competitive binding experiments showed that EPA-EA had a 4- to 16.5-fold weaker binding to CB₁ compared to AEA (Table 1) (28, 59, 60).

Years later, EPA-EA was isolated from animal tissues, compared to AEA and DHA-EA, and levels were significantly lower or undetectable at basal levels (43). Additionally, when piglets were fed a fish oil diet, phospholipids containing esterified EPA significantly increased, while EPA-EA levels were undetectable in the brain, jejunum and liver (31). Yet, in adipose tissue, mice fed with omega-3 oil, levels of EPA-EA increased (33). Lastly, EPA-EA has also been detected in human milk, similar to DHA-EA (36) (Table ST1). Together, these findings further support that endogenous levels can be altered through dietary interventions and the levels of EPA-EA vary by tissue type.

2.1. Role of EPA-EA in Inflammation

Since EPA has shown to possess anti-inflammatory properties, the anti-inflammatory effects of EPA-EA was studied in adipocytes and myoblasts. In 3T3-L1 adipocytes, 10 to 50 μ M of EPA supplementation led to a 3.5- to 10.9-fold increase of EPA-EA. When LPS-stimulated adipocytes were treated with EPA-EA, levels of pro-inflammatory cytokines, IL-6 and MCP-1, were reduced by 50% at 100 nM and 10 nM, respectively (Table 3). Moreover, LPS-stimulation increased EPA-EA levels several fold higher than DHA-EA, possibly due to the higher endogenous levels of esterified EPA than DHA (47, 61).

In C₂C₁₂ myoblasts treated with EPA-EA, the expression of CB receptors and glucose-uptake receptors were increased. Additionally, EPA-EA-induced CB receptor expression was investigated for regulating insulin sensitivity via activation of the mitogen-activated protein kinase (MAPK) pathway. Interestingly, when C₂C₁₂ myoblasts were supplemented with EPA-EA, p38 MAPK phosphorylation was altered. Additionally, N-acyl phosphatidylethanolamine phospholipase mRNA levels increased with EPA-EA supplementation (Table 3) (55). Collectively, these findings propose that EPA-EA may be indirectly mediating its anti-inflammatory effects through increased levels of other fatty acid-derived eCBs. Ultimately, they may aid in the reduction of pro-inflammatory cytokines and increase insulin sensitivity through the MAPK pathway.

2.2. Role of EPA-EA in Cancer

EPA-EA has been shown to inhibit prostate cancer cell growth and induce cell death of LNCaP and PC3 cell lines. In these cells, EPA-EA was determined to be an agonist to CB₁ and CB₂ (Table 1). Similar to DHA-EA, the anti-proliferative effects of EPA-EA were significantly reduced in the presence of FAAH inhibitors (18). Additionally, in breast cancer cells (MCF-7), EPA-EA inhibited proliferation in a dose-dependent manner with an IC₅₀ value of 1.5 μ M (50). EPA-EA also demonstrated to activate PPAR γ , which plays a key role in the autophagy process (Table 3). When PPAR γ is activated, an autophagy process occurs

in which activation of beclin-I is observed. When the autophagy process becomes inhibited, beclin-I interacts with Bcl-2. Remarkably, EPA-EA reduced the beclin-I and Bcl-2 interaction and ultimately inhibited the AKT-mTOR signaling pathway, which plays a key role in the autophagy and apoptotic processes of the cell cycle (50).

2.3. Metabolism of EPA-EA and Role of Metabolites

EPA-EA is metabolized by CYP450 to form corresponding epoxides that are found endogenously in rat tissues (Figure 2B). Specifically, CYP2J2, metabolized EPA-EA into five regioisomers called epoxyeicosatetraenoic acid-ethanolamides (EEQ-EAs). Of the five regioisomers produced, 17,18-EEQ-EA was the predominant metabolite formed (Table 3). Similar to 19,20-EDP-EA, 17,18 EEQ-EA significantly reduced pro-inflammatory NO and IL-6, whereas anti-inflammatory IL-10 production significantly increased in microglia cultures. Additionally, 17,18-EEQ-EA was also determined to be three- and six-fold more susceptible to FAAH and soluble epoxide hydrolase (sEH) hydrolysis, respectively, when compared to 19,20-EDP-EA. EPA-derived metabolites are more susceptible to breakdown compared to DHA-derived metabolites, which could be due to their structural differences. Interestingly, 17,18 EEQ-EA also inhibited platelet aggregation regulated vasodilation and angiogenesis, as well as activated CB₁ and CB₂ with an EC₅₀ value of 18.5 nM and 1.4 nM, respectively (Table 1) (19).

2.4. Summary of DHA-EA and EPA-EA

In conclusion, endogenous DHA and EPA are non-oxidatively converted to DHA-EA and EPA-EA, respectively. These omega-3 eCBs are precursors to more potent bioactive molecules that interact through CB-independent and -dependent-pathways. For instance, DHA-EA and EPA-EA can undergo further downstream modifications through the canonical oxygenase pathway involving COX, LOX, and CYP450. The mechanism of action of these newly formed metabolites remains to be investigated. Thus far, DHA-EA and EPA-EA possess anti-proliferative as well as anti-inflammatory properties and may be key modulators of the inflammatory cascade. Additionally, DHA-EA and EPA-EA possess some affinity to CB and non-CB receptors (Table 1) (19, 20, 62). Although we focus on the nutritionally relevant omega-3 derived eCBs, from DHA and EPA, it is important to highlight reports that have demonstrated that similar analogues, such as α -linolenoyl ethanolamine (ALA-EA), possess pharmacological significance through activation of transient receptor potential vanilloid 1 (TRPV1) and not CB₁ (28, 63). Therefore, there is a large class of omega-3 fatty acid derived eCB-like molecules that can modulate cell physiology through other non-CB receptors.

3. Omega-3 fatty acid DHA and EPA glycerol (DHG and EPG)

Another class of potential omega-3 eCBs with structural similarities to 2-AG have also been discovered. DHA-derived, docosahexaenoyl glycerol (DHG) and EPA-derived, 2-5,8,11,14,17-eicosapentaenoyl glycerol (EPG), have a glycerol ester linkage (Figure 1C). Both 2-DHG and 2-EPG were first reported in a study investigating 2-AG analogues. Since 2-AG induced a rapid, increase in intracellular free calcium levels, the effects of structural analogues were also assessed. When compared, 2-AG and 2-EPG both induced cAMP

levels, that are partially mediated by CB₁, with similar agonist activities. However, 2-DHG showed weak agonist activity at the CB₁ (21). These initial studies set the precedence for investigating the endogenous production of 2-DHG and 2-EPG as well as their bioactive roles.

It is important to note that 2-AG isomerizes to 1-AG and is also hydrolyzed by active MAGL to form AA. Similarly, 2-DHG and 2-EPG are not thermodynamically stable and can isomerize to 1-DHG and 1-EPG. Furthermore, they are susceptible to become hydrolyzed to form DHA and EPA. Therefore, the effects observed with 2-DHG or 2-EPG might be partially be due to the unesterified DHA and EPA that is formed after the corresponding monoacyl-glycerol cleavage.

3.1. Endogenous Levels of 2-DHG and 2-EPG

Both 2-EPG and 2-DHG were found in mouse embryonic stem cells (mES) and embryoid bodies. In mES cells, there were comparable concentrations of 2-DHG and 2-EPG (43). Additionally, a high omega-3 and low omega-6 diet in humans produced a significant increase in 2-DHG levels, whereas 2-AG levels decreased. Furthermore, EPG and DHG levels have shown to increase in human milk upon maturation (Table ST1) (36). Strikingly, an increase in 2-DHG was correlated with a decrease in pain (Table 3 & ST1) (56). Despite the detection of 2-DHG and 2-EPG, the biological and physiological significance were not evident.

3.2. Bioactivity of 1-DHG

2-AG is one of the most well studied eCB. However, it isomerizes spontaneously into 1-AG under physiological conditions and is considered a potential mechanism of its deactivation (64–66). In fact, it has been reported that commercially available 2-AG contains approximately 10% 1-AG, thus the isomerization process is inevitable in biochemical research assays (66). Hence, testing of the potential effects of 2-AG is undermined due to the rate of isomerization and the chemical instability of these molecules (65, 66). 1-DHG is structurally similar to 2-DHG, containing a DHA attached to the sn-1 instead of the sn-2 position of the glycerol molecule via an ester bond. 1-DHG is also referred to as monoacylglyceride docosahexaenoic acid (MAG-DHA) (67). Its effects in pulmonary arterial hypertension (PAH) and cystic fibrosis have recently been investigated (Table 2).

PAH is a disease characterized by a progressive increase in pulmonary pressure and vascular resistance (68). It has been previously demonstrated that CYP450s in the lungs, including CYP2J2, CYP2C8 and CYP2C9, can metabolize AA, EPA and DHA into oxygenated eicosanoids, which have been reported to modulate vascular and airway smooth muscle tone (68–70). Thus, the ability for 1-DHG to inhibit vasoconstriction was first investigated using human pulmonary cells. In these studies, 1-DHG was found to reduce vasoconstriction in a dose-dependent manner, with an EC₅₀ of 1.25 μ M. In addition, 1-DHG was able to reduce tension in human pulmonary arteries by 50% at 3 μ M. Interestingly, this tension was further reduced to 95% in the presence of a Rho-kinase inhibitor, H-1152 (67). Since 1-DHG reduced vasoconstriction modulated by Rho-kinase, further studies showed that 30 μ M of 1-DHG was able to inhibit RhoA activity, a known activator of Rho-kinase (68). Furthermore,

it was revealed that 1-DHG treatment reduced phosphorylation of downstream members of the Rho-kinase pathway (Table 2) (67, 68). Thus 1-DHG may represent a new pharmacological alternative in modulating vasoconstriction in patients with pulmonary hypertension.

Homeostatic response caused by omega-3 eCBs and their metabolites are not limited to cancer, inflammation or pain, as these molecules can act broadly in different disease states as inflammation is common etiology in many diseases. For instance, cystic fibrosis is the most common autosomal, recessive, inherited disease with fatal outcome in Caucasians, and is characterized by persistent lung infections and losing the ability to breathe over time (71). Previously, cystic fibrosis patients have reported a fatty acid deficiency with an imbalance between omega-3 and omega-6 levels in their blood lipids (72, 73). Since omega-3 fatty acid supplements were found to reduce inflammation in cystic fibrosis patients, the effects of 1-DHG were investigated utilizing lung adenocarcinoma Calu-3 cells. At 3 μ M, 1-DHG treatment was found to increase the mean DHA/AA ratio with at least a two-fold increase in DHA/AA ratio from control. Moreover, 1-DHG treatment also led to a reduction of pro-inflammatory markers, such as IL-6 and IL-8, induced by LPS. Notably, docosaepentaenoic glycerol (DPG) and EPG showed a more effective reduction (Table 2) (74). This study was the first to demonstrate the anti-inflammatory properties of 1-DHG and its potential in the management of cystic fibrosis. While there are some studies reported for 1-DHG, there are no current reports on 2-DHG.

3.3. Bioactivity of 1-EPG

Similar to DHA, EPA also has a monoacylglyceride derivative known as MAG-EPA or 1-EPG (Figure 1C). Previously, 1-EPG has shown to be anti-inflammatory through reduction of pro-inflammatory NF- κ B and COX-2. Use of an asthma model has demonstrated that 1-EPG treatment reduced the reactivity of airway smooth muscles with an EC₅₀ value of 0.77 nM. Interestingly, 1-EPG significantly reduced calcium sensitivity and decreased the omega-6/omega-3 ratio in the asthma model (Table 3) (75). These findings directly support the previous findings that 1-EPG caused these effects, as omega-6 fatty acid levels are positively correlated with asthmatic symptoms. In related studies, the effects of 1-EPG on airway hyper-responsiveness were investigated. In guinea pig models, 1-EPG reduced tracheal ring tension with an IC₅₀ = 1.03 μ M. It was further reported that an EPA metabolite, 17,18-epoxyeicosatetraenoic acid (17,18-EEQ) had a greater effectiveness compared to its precursor 1-EPG (76). The findings of this study suggest that 1-EPG may have potential in the treatment of respiratory-related diseases, possibly through its metabolism to the highly bioactive 17,18-EEQ metabolite (76).

Since omega-3 supplementation causes a decrease in the production of inflammatory cytokines involved in ulcerative colitis pathogenesis, the effects of 1-EPG in ulcerative colitis were also investigated. In a colitis rat model, orally administered 1-EPG was found to reduce the severity of colitis, further suggesting that 1-EPG may prevent the acceleration of ulcerative colitis (77). To investigate the potential mechanism of 1-EPG in colitis, pro-inflammatory cytokines were measured. In colon tissue, an 81% reduction of NF- κ B was observed with 1-EPG treatment. As in previous studies, TNF- α , IL-1 β , IL-6 and COX-2

expression levels were also decreased when treated with 1-EPG, suggesting that 1-EPG may act on a transcriptional level. Collectively, these studies suggest that 1-EPG is anti-inflammatory in several models, including asthma, airway hyper-responsiveness, and ulcerative colitis.

It is known that DHA and EPA have beneficial effects in cancer models *in vitro* and *in vivo* (78). Compared to 1-DHG, 1-EPG decreased cell growth of human colorectal carcinoma cells, where apoptosis was even induced in a dose-dependent manner (0.3 μ M to 10 μ M) by 1-EPG (79). *In vivo* mouse model studies demonstrated that 1-EPG reduced tumor sizes by 3-fold after daily administration. Moreover, 1-EPG treatments reduced VEGF and HIF-1 α expression levels in tumors, both of which are commonly overexpressed in many cancers. 1-EPG downregulated the gene products of NF- κ B, VEGFR, and AKT within the colorectal carcinoma study, all of which are associated with angiogenesis, metastasis and proliferation of tumors (Table 3) (79). While these effects were observed in mice, a study is currently in progress to investigate whether the 1-EPG in cell cultures translates in humans. The effects of 1-EPG on prostate cancer on randomized, double-blind, placebo-controlled trial is currently being conducted as well, highlighting the potential far-reaching implications of eCB-based therapeutics (80).

4. N-acyl neurotransmitter derivatives of DHA and EPA: Dopamine and Serotonin

Neurotransmitters enable neurotransmission in the brain and are synthesized from amino acids precursors. Biogenic amines such as monoamine neurotransmitters and catecholamine neurotransmitters are crucial for transmitting signals across a chemical synapse from one neuron to the target cells. Recently, DHA and EPA derived conjugates of monoamine neurotransmitters have become of interest due to the potential dual physiological effects of omega-3 fatty acids and neurotransmitters. Herein, we discuss omega-3 derived N-acyl conjugates of neurotransmitters and their potential roles in immunomodulation and neurodegeneration.

4.1. Expression and biological effects of DHA-Dopamine and EPA-Dopamine (DHA-DA or EPA-DA)

N-arachidonoyl dopamine (NADA) was first reported as a novel CB₁ ligand (81) (Figure 1D). Similar to NADA, DHA-dopamine (DHA-DA) is of interest due to its potential physiological properties that are linked to brain synapses and retinal neuroprotection (82). The synthesis and biological effects of omega-3 derived neurotransmitters, such as DHA-DA has been shown to possess analgesic and antioxidative effects (Table 2) (83, 84). In addition, DHA-DA decelerates the development of Parkinson's disease symptoms and recently has shown to be anti-inflammatory (85). In rodent macrophages and microglia cells, DHA-DA dose-dependently, at 10 nM to 2.5 μ M, suppressed the production of four pro-inflammatory markers: NO, MCP-1, macrophage-inflammatory protein 3 α and IL-6. Other immunomodulatory effects by DHA-DA is suppressing levels of prostaglandin E₂ (PGE₂), a COX-2 product that promotes inflammation, pain and fever. Notably, PGE₂ was suppressed dose dependently by DHA-DA up to 75% at 1 μ M. Similarly, COX-2 transcript levels were

also dose-dependently decreased by DHA-DA (86). However, DHA-DA did not affect NF- κ B, a transcriptional regulator of COX-2. Notably, similar findings of DHA-DA in macrophages were consistent in BV-2 microglia cells, however COX-2 gene expression in LPS-activated microglial cells were unaffected (86). Collectively, this evidence suggests that DHA, the most abundant fatty acid in the brain, and dopamine, an important neurotransmitter in the brain, together form a lipid mediator conjugate that can modulate neuroinflammation.

In human breast cancer cell lines, DHA-DA and EPA-DA has been reported to induce cell death through its interaction with PPAR γ . Both DHA-DA and EPA-DA dose-dependently reduced cell viability in MCF-7, SKBR3 and MDA-MB-231 cell lines. In MCF-7, SKBR3 and MDA-MB-231, DHA-DA dose- and time-dependently reduced cell viability in all cell lines with IC₅₀ values of 25, 20 and 6 μ M, respectively. Furthermore, DHA-DA did not appear to influence proliferation of non-tumorigenic breast MCF-10A cells. Notably, this data combined with the IC₅₀ values of tumorigenic breast cancer cell lines indicate DHA-DA has greater effects on more aggressive cell types than others. Increased expression of PPAR γ was measured and further studies demonstrated that activation of PPAR γ by DHA-DA and EPA-DA mediate the antiproliferative effects in cancer cells. Notably, DHA-DA and EPA-DA also upregulated autophagic protein, Beclin-1, expression in these cells (87). Together, this suggests that DHA-DA and EPA-DA activation of PPAR γ may have potential in adjuvant therapeutic interventions in breast cancer.

4.2 Expression and Biological effects of DHA-Serotonin and EPA-Serotonin (DHA-5HT or EPA-5HT)

It has been demonstrated that the endogenous production of N-acyl amides and serotonin levels are increased in intestinal tissues with omega-3 supplementation (88). In the first study, the serotonin conjugate of DHA, DHA-serotonin (DHA-5HT), significantly inhibited pro-inflammatory mediators, IL-6 and PGE₂, at 2.5 μ M in LPS-stimulated murine macrophages (Table 2). Similar to DHA-DA, gene expression of COX-2, IL-6 and NO synthase 2 were all suppressed by DHA-5HT at the transcriptional level. Furthermore, DHA-5HT treatment led to downregulation of co-stimulatory molecules needed for T cell activation, demonstrating that DHA-5HT reduces gene expression of mediators involved in inflammatory T cell responses. Moreover, microarray analysis found DHA-5HT to reduce the gene expression of metalloproteinases, chemokines and pro-inflammatory interleukins such as IL-12b, IL-23a, IL-6, and IL-1 β . Notably, these observations were consistent in LPS-stimulated RAW264.7 cells in which DHA-5HT treatments inhibited migration of macrophages by 52% and 77%, at 1 μ B and 2 μ M, respectively (Table 2) (89). DHA-5HT also reduced the production of MCP-1 and CCL-20, a chemokine released by pathogenic pro-inflammatory T helper 17 (Th17) cells, to that of DHA-DA (86). Collectively, this evidence suggests that DHA-5HT may have therapeutic potential for patients with intestinal immune-dysregulation of inflammatory T cells.

Recently the presence and effects of DHA-5HT in human tissues was demonstrated. LC-MS/MS analysis, found that both DHA-5HT and EPA-5HT were detected in addition to other N-acyl-5HT. Notably, out of the six N-acyl serotoninins detected, DHA-5HT was most

effective at suppressing pro-inflammatory cytokine IL-17 release in peripheral blood mononuclear cells (PBMCs) in a dose-dependent manner. DHA-5HT treatments decreased IL-17 production by PBMCs by 80% and 39% at 10 μ M and 1 μ M, respectively. Interestingly, DHA and serotonin separately did not influence IL-17 release, suggesting that the N-acyl serotonin moiety is vital for this physiological effect. Measuring CCL-20 production helped probe the immunomodulatory effects of DHA-5HT. Similar to IL-17, CCL-20 production was dose-dependently and significantly attenuated, by 39.5% and 45.95%, at 5 and 10 μ M treatments, respectively (90). Hence, DHA-5HT regulates the production of two important Th17 mediators involved in pathogenesis of intestinal diseases and is a class of immunomodulatory omega-3 derived lipid mediators.

The mechanism of action of DHA-DA and DHA-5HT still remains unknown. However, similar conjugates such as, ALA-DA and ALA-5HT, can activate CB and TRPV1 receptors and inhibit FAAH degradation of AEA (84, 91). Thus, the potential mechanism of action of DHA-DA, DHA-5HT and the EPA analogues may follow similar receptor interactions. As eCBs also act through TRPV1 (92–94), they are termed as “endovanilloids”. In addition to mediating the pain associated from stimuli, TRPV1 exhibits pro- and anti-inflammatory effects (95–98). Recently, it has been postulated that there is a crosstalk between CB₁ and TRPV1 receptors, which are co-localized in dorsal root ganglion. Therefore, the ECS and TRPV1 axis provides a promising target to develop pain and inflammation therapeutics.

5. Concluding Remarks

In the past few decades, a significant amount of work was performed to understand the role of omega-3 and omega-6 derived eCBs and their derivatives the overall physiology of the body. Despite structural similarities, common pathways for formation and enzymatic degradation, eCBs are intriguing lipid signaling molecules with varied interactions with several different types of receptors leading to varied physiological roles. Though previous studies focus on cannabinoid receptor activation, it is important to acknowledge that omega-3 and omega-6 eCBs could possibly activate other receptors as they are multi-functional molecules. Therefore, systems-based approaches are required to fully characterize these molecules with respect to receptor interactions and downstream signaling. Their on-demand signaling capabilities could suggest that they act at multiple receptors in tandem until they resolve the homeostatic challenge or are converted to new molecules with altered bioactivity through different pathways including eicosanoid synthesizing enzymes. All of these create possibilities for multiple modes of action of eCBs, create new areas of cross-talk to investigate. As shown, omega-3 and omega-6 fatty acid eCBs have demonstrated metabolism by LOX-, COX- and CYP450-mediated pathways, yet only LOX and CYP450 metabolites have been reported for DHEA and only CYP450 metabolites for EPEA (Figure 2). Thus, the physiological function of the LOX and CYP450 generated metabolites remains to be elucidated further to understand how these lipids modulate cell signaling under diseased and non-diseased states.

We must continue to focus on the influence of naturally produced omega-3 eCB derivatives to better understand how they display such ubiquitous biological roles. Referencing the molecular origins and fates of these molecules while discussing their physiological roles is

critical in driving future research surrounding omega-3-derived eCBs. Many of the studies mentioned in this review limit our understanding of these molecules, as this basic characterization is biased towards measuring just CB receptor interaction and direct inflammatory responses. Hence, more research is needed to describe these molecules across multiple disease models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

Δ^9-THC	Δ^9 -tetrahydrocannabinol
GPCR	G-Protein coupled receptor
CB₁	cannabinoid receptor 1
CB₂	cannabinoid receptor 2
eCB	endocannabinoid
ECS	endocannabinoid system
cAMP	cyclic adenosine monophosphate
AA	arachidonic acid
AEA	anandamide
2-AG	2-arachidonoyl-glycerol
DHA	docosahexaenoic acid
2-DHG	2-docosahexaenoyl-glycerol
DHA-EA	docosahexaenoyl ethanolamide
DHA-DA	DHA-Dopamine
DHA-5HT	DHA-Serotonin
EPA	eicosapentaenoic acid
EPA-EA	eicosapentaenoyl ethanolamide
EPG	eicosapentaenoyl-glycerol

EPA-DA	EPA-Dopamine
EPA-5HT	EPA-Serotonin
COX	cyclooxygenase
LOX	lipoygenase
FAAH	fatty amide hydrolase
sEH	soluble epoxide hydrolase
MAGL	monoacylglycerol lipase

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Highlights

- Omega-3 fatty acid endocannabinoids are derived from omega-3 fatty acids and are found endogenously in the body.
- Despite similar structural features, omega-3 fatty acid endocannabinoids and their derivatives demonstrate a perplexing diversity in function and receptor interactions that warrants further molecular characterization.

Figure 1. Omega-3 endocannabinoids and derivatives.

Chemical Structures of (A) Omega-3 fatty acids, Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) and N-acyl amide derivatives that have been identified in literature. (B) Docosahexaenoyl ethanolamide (DHA-EA), Eicosapentaenoyl ethanolamide (EPA-EA), (C) 1-Docosahexaenoyl-glycerol (1-DHG), 1-Eicosapentaenoyl-glycerol (1-EPG), (D) DHA-Dopamine (DHA-DA), EPA-Dopamine (EPA-DA), DHA-Serotonin (DHA-5HT) and EPA-Serotonin (EPA-5HT).

Figure 2. Oxidation of Omega-3 endocannabinoids (DHA-EA and EPA-EA) by the LOX and CYP450 pathway.

(A) For the LOX pathway, oxidation can occur once and metabolites formed can undergo additionally oxidation or reduction to form seven DHA-EA-LOX metabolites; 17-hydroperoxydocosahexaenoyl ethanolamide (17-HpDHA-EA), 17-hydroxy-4Z,7Z,10Z,13Z, 15E,19Z-docosahexaenylethanolamide (17-HDHA-EA), 10,17-dihydroxydocosahexaenoyl ethanolamide (10,17-diHDHA-EA), 7,17-dihydroxydocosahexaenoyl ethanolamide (7,17-diHDHA-EA), 4,17-dihydroxydocosahexaenoyl ethanolamide (4,17-diHDHA-EA), 13-hydroxy-16(17)-epoxy-docosapentaenylethanolamide (13-HEDPEA) and 15-hydroxy-16(17)-epoxy-docosapentaenylethanolamide (15-HEDPEA). (B) The CYP450 pathway oxidizes DHA-EA and EPA-EA into six epoxydocosapentaenoic acid-ethanolamide

(EDP-EA) and five epoxyeicosatetraenoic acid-ethanolamide (EEQ-EA) regioisomers, respectively.

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Table 1.

Cannabinoid Receptor Binding and Activation of DHA-EA, EPA-EA and oxygenated metabolites

Compound	CB ₁ EC ₅₀ values (nM)	CB ₂ EC ₅₀ values (nM)	References
AEA	0.7 ^a	1352 ^a	(19)
	39.2 ± 5.7 ^b	ND ^b	(28)
	543 ± 83 ^b	ND ^b	(12)
	160 ± 13 ^c	ND ^c	
	89 ± 10 ^b (with PMSF)	ND ^b	(60)
	5400 ± 1600 ^b	ND ^b	
DHA-EA	1044 ^a	305 ^a	(19)
	9.8 ^a	ND ^a	(20)
	12200 ± 520 ^b	ND ^b	(12)
	6000 ± 800 ^c		
	324.1 ± 9.2 ^b	ND ^b	(28)
19,20 EDP-EA	108 ^a	280 ^a	(19)
10,17-diHDHA-EA	ND ^a	3.9 ^a	(20)
15-HEDPEA	ND ^a	1 ^a	(20)
EPA-EA	0.1 ^a	2.1 ^a	(19)
	1470 ± 500 ^b (with PMSF)	ND ^b	(60)
	162.3 ± 13.6 ^b	ND ^b	(28)
17,18 EEQ-EA	18.5 ^a	1.4 ^a	(19)

Abbreviations: N-docosahexaenylethanolamine (DHA-EA), eicosapentaenyl ethanolamide (EPA-EA), epoxydocosapentaenoic acid-ethanolamide (EDP-EA), epoxyeicosatetraenoic acid-ethanolamide (EEQ-EA), dihydroxydocosahexaenyl ethanolamide (diHDHA-EA), hydroxy-epoxy-docosapentaenyl ethanolamide (HEDPEA) and not determined (ND).

^aExperiments were performed with cell cultures transfected with CB receptors

^bExperiments were performed with displacement of CB receptor agonist

^cExperiments were measured by inhibition of cAMP

Table 2.**Biological Effects of DHA-derived eCBs and Derivatives**

<i>Effects of DHEA in Brain</i>	References
9.5-fold increase in piglet brain from DHA supplementation	(30)
Synaptic activity induced in hippocampal neurons	(39)
Neurite growth increased 10-fold greater than DHA	(39)
50 to 100-fold more effective than DHA in differentiating NSCs	(44)
Phosphorylation of PKA substrates increased	(44)
Ethanol impairment reversed	(45)
Cellular cAMP production increased	(45)
GPCR activation increased	(45)
Reversed cAMP reduction caused by ethanol (25mM)	(45)
Promoted axon development	(46)
Negatively regulated sonic hedgehog signaling in cortical neurons	(46)
Increased cortical neuron growth	(46)
Inhibited sonic hedgehog induced signaling	(46)
<i>Effects of DHEA in adipose, peritoneum and pain</i>	
Reduced IL-6 and MCP-1 levels in LPS-stimulated adipocytes	(47)
Causes negative correlation in size of adipocytes	(33)
Reduced nitric oxide production and MCP-1 levels in peritoneal macrophages and RAW264.7 cells	(48)
Increases glucose up-take, CB ₁ , CB ₂ and NAPE-PLD expression in C ₂ C ₁₂ myoblasts	(55)
Reduced headache severity by High ω -3/Low ω -6 diet	(56)
<i>Effects of DHEA in cancer</i>	
Induced death of LNCaP and PC3 prostate cancer cells to androgen	(18)
Inhibits proliferation of MCF-7 breast cancer cells	(50)
Yield reduction of AKT-mTOR pathway	(50)
<i>Effects of DHEA metabolized by lipoxygenase (LOX)</i>	
Reduced migration of PMN chemotaxis by IL-8	(20)
Activates CB ₂ receptors (10,17-diDHEA & 15-HEDPEA)	(20)
Decreased platelet-leukocyte aggregation by 30%–40%	(20)
<i>Effects of Docosahexaenoyl Ethanolamide Epoxides (EDP-EA)</i>	
Significantly reduced pro-inflammatory nitric oxide and IL-6	(19)
Induced anti-inflammatory cytokine production in primary microglial cultures	(19)
<i>Effects of Monoacylglyceride Docosahexaenoic Acid (1-DHG)</i>	
Reduced vasoconstriction from human pulmonary tissues	(68)
Reduced arterial tension comparably to H-1152 by 50%	(68)
Reduced RhoA activity by lowering coupling to GTP	(68)
Reduced phosphorylation of Rho-kinase, MYPT-1 and MLC	(67, 99)

<i>Effects of DHEA in Brain</i>	References
Reverse hypertension in human pulmonary arteries	(67, 99)
Reduced expression of VEGF	(67, 99)
Reduced inflammatory markers in cystic fibrosis induced by LPS	(74)
<i>Effects of Docosahexaenoic Acid Dopamine (DHA-DA)</i>	
Decelerated development of Parkinson's disease symptoms	(85)
Elicits antioxidative properties under oxidative stress conditions	(85)
Suppressed nitric oxide production	(85)
Reduced pro-inflammatory markers, MCP-1, CCL-20 and IL-6	(85)
Suppressed PGE ₂ by 75%	(85)
Reduced breast cancer cell viability	(87)
Decreases COX-2 transcript levels	(77)
<i>Effects of Docosahexaenoic Acid Serotonin (DHA-5HT)</i>	
Inhibited IL-6 by 75% in LPS-stimulated RAW262.7 cells	(89)
Inhibited PGE ₂ in LPS-stimulated RAW262.7 cells	(89)
Suppressed pro-inflammatory mediator transcript levels	(89)
Downregulated pro-inflammatory IL's and co-stimulatory T cell molecules	(89)
Inhibited migration of RAW264.7 cells	(89)
Suppressed IL-17 in PBMC's by 80%	(90)

Table 3.

Biological effects of EPA-derived eCBs and Derivatives

Effects of Eicosapentaenoyl Ethanolamide (EPEA)	References
Reduced IL-6 levels in LPS-stimulated 3T3-L1 adipocytes	(47)
Reduced MCP-1 production by 50% in LPS-stimulated 3T3-L1 cells	(47)
Inhibited MCF-7 cancer cell proliferation	(50)
<i>Effects of Eicosapentaenoyl Ethanolamide Epoxides (EEQ-EA)</i>	
Reduced pro-inflammatory nitric oxide and IL-6	(19)
Induced production of anti-inflammatory IL-10 in microglia	(19)
<i>Effects of Monoacylglyceride Eicosapentaenoic Acid (1-EPG)</i>	
Dose administration reduced reactivity of airway smooth muscles	(75)
Reduced expression NF κ B and COX-2	(75)
Reduced tracheal ring tension induced by U-46619	(76)
Reduced severity of DSS-induced colitis	(77)
Reduced pro-inflammatory cytokines TNF α , IL-1 β , and IL-6	(77)