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Cytochrome P₄₅₀ epoxygenase pathway of polyunsaturated fatty acid metabolism

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Abstract

Polyunsaturated fatty acids (PUFA) are oxidized by cytochrome P450 epoxygenases to PUFA epoxides which function as potent lipid mediators. The major metabolic pathways of PUFA epoxides are incorporation into phospholipids and hydrolysis to the corresponding PUFA diols by soluble epoxide hydrolase. Inhibitors of soluble epoxide hydrolase stabilize PUFA epoxides and potentiate their functional effects. The epoxyeicosatrienoic acids (EETs) synthesized from arachidonic acid produce vasodilation, stimulate angiogenesis, have anti-inflammatory actions, and protect the heart against ischemia-reperfusion injury. EETs produce these functional effects by activating receptor-mediated signaling pathways and ion channels. The epoxyeicosatetraenoic acids synthesized from eicosapentaenoic acid and epoxydocosapentaenoic acids synthesized from docosahexaenoic acid are potent inhibitors of cardiac arrhythmias. Epoxydocosapentaenoic acids also inhibit angiogenesis, decrease inflammatory and neuropathic pain, and reduce tumor metastasis. These findings indicate that a number of the beneficial functions of PUFA may be due to their conversion to PUFA epoxides.

Keywords

arachidonic acid (AA); epoxyeicosatrienoic acid (EET); eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); epoxyeicosatetraenoic acid (EpETE); epoxydocosapentaenoic acid (EpDPE)

1. Introduction

Oxidation of PUFA by cytochrome P450 (CYP)¹ epoxygenases was first detected when rat kidney or liver microsomes were incubated with arachidonic acid (AA) in the presence of NADPH and O₂ [1,2]. The epoxyeicosatrienoic acids (EET) synthesized from AA were initially observed to function as lipid mediators in the cardiovascular and renal systems [3–7]. EETs are metabolized by many tissues [1,8,9], and recent findings indicate that they have effects on angiogenesis, proliferation, inflammation, pain and myocardial preconditioning

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[10–15]. CYP epoxygenases also oxidize linoleic acid (LA), the 18-carbon ω -6 PUFA, to epoxyoctadecenoic acids (EpOME), and the ω -3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to epoxyeicosatetraenoic acids (EpETE) and epoxydocosapentaenoic acids (EpDPE), respectively [2]. Like EETs, the ω -3 PUFA epoxides function as lipid mediators. They have potent anti-arrhythmic actions, protect the heart against ischemia-reperfusion injury and are analgesic on inflammatory and neuropathic pain. Epoxydocosapentaenoic acids also inhibit angiogenesis and have been observed to reduce tumor metastasis. They appear to be much more potent than EETs in reducing arrhythmias, more potent in reducing blood pressure, and even somewhat more potent in reducing inflammation and pain [16–18].

2. Arachidonic acid epoxidation

Epoxidation of AA has been studied extensively in the endothelium, the main source of EETs in the vascular system. Endothelial cells express CYP2J2 and CYP2C9, and EET production is stimulated by exposure of these cells to vasodilators like bradykinin and acetylcholine, or by shear stress [19–21]. As illustrated in Fig. 1, AA stored in phospholipids is hydrolyzed by a Ca^{2+} -activated phospholipase A₂ (PLA₂) and oxidized by a CYP epoxygenase to form EETs. Although CYP epoxygenases can epoxidize each of the four double bonds of AA, most epoxygenases produce appreciable amounts of only one or two EET regioisomers. 11,12- and 14,15-EET account for 67–80% of the EETs synthesized by five purified and reconstituted CYP epoxygenases, including CYP2C8, CYP2C9 and CYP2J2, and 11,12- and 14,15-EET are the main EET regioisomers produced by most mammalian tissues [1,22].

Each EET regioisomer consists of two enantiomeric forms, *R/S* and *S/R*. The enantiomeric distributions are different for each CYP epoxygenase, and two regioisomers synthesized by the same CYP epoxygenase often have different enantiomeric distributions [1,22].

Enantiomeric dependence has been observed for some EET functions. For example, 11,12-EET-induced relaxation of rat renal microvessels and activation of renal vascular smooth muscle large-conductance Ca^{2+} -activated K^{+} (BK_{Ca}) channels are mediated only by 11(*R*), 12(*S*)-EET [23], whereas the activation of cardiac K_{ATP} channels is mediated only by 11(*S*), 12(*R*)-EET [24].

3. Epoxidation of linoleic acid and ω -3 PUFAs

Fig. 2 illustrates the structures of the major epoxide products synthesized by CYP epoxygenases from LA, EPA and DHA. The main LA-derived epoxide produced by leukocytes, 9,10-EpOME, is called leukotoxin because it becomes cytotoxic and produces

¹Abbreviations: CYP, cytochrome P450; AA, arachidonic acid; EET, epoxyeicosatrienoic acid, LA, linoleic acid; EpOME, epoxyoctadecenoic acid; EPA, eicosapentaenoic acid; EpETE, epoxyeicosatetraenoic acid; DHA, docosahexaenoic acid; EpDPE, epoxydocosapentaenoic acid; PLA₂, phospholipase A₂; BK_{Ca}, large-conductance Ca^{2+} -activated K^{+} channels; sEH, soluble epoxide hydrolase; DHET, dihydroxyeicosatrienoic acid; FABP, cytosolic fatty acid binding protein; EH3, epoxide hydrolase ABHD9; AUDA, 12-(3-adamantan-1-yl-ureido)dodecanoic acid; *t*-AUCB, *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid; TUPS, 1-(1-methylsulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)urea; 8-HUDE, 12-(3-hexylureido) dodec-8-enoic acid; MS-PPOH, *N*-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide; 14,15-EEZE, 14,15-epoxyeicosa-5(*Z*)-enoic acid; PPAR, peroxisome proliferator-activated receptor; PKA, protein kinase A; TRP, transient receptor potential channel; ENaC, epithelial sodium channel; VACC, volume-activated chloride channel; K_{ATP}, ATP-dependent potassium channel

multiple organ failure and respiratory distress when it is hydrolyzed by an epoxide hydrolase to 9,10-dihydroxyoctadecaenoic acid [25]. Much higher concentrations of 9,10-dihydroxyoctadecaenoic acid than other PUFA-derived diols are present in the plasma of healthy American adults, which may reflect the high levels of linoleic acid in the Western diet [26]

CYP epoxygenases preferentially oxidize the ω -3 double bond of EPA and DHA. The most abundant DHA regioisomer synthesized by microsomes and by 15 human recombinant CYPs, including CYP2C8 and CYP2J2, is 19,20-EpDPE. Likewise, the most abundant EPA regioisomer synthesized by these CYPs is 17,18-EpETE [2,27,28]. An exception is CYP2C9 which oxidizes EPA mainly to 14,15-EpETE and DHA to 10,11-EpDPE [17]. EPA and DHA compete with AA for hepatic and renal microsomal epoxigenase activity and for recombinant CYP2C9, CYP1A2, CYP2C8 and CYP2J2, and thus, EET synthesis is decreased in the presence of these ω -3 PUFAs [28–30].

The CYP epoxide profiles in plasma and tissues can be modified by dietary ω -3 PUFA supplementation. When healthy human adults were supplemented with 4 g per day of ω -3 PUFA, the plasma 19,20-EpDPE and 16,17-EpDPE contents doubled, and there was a 4-fold increase in 17,18-EpETE and 14,15-EpETE [26]. Likewise, substantial increases in 19,20-EpDPE, 16,17-EpDPE and 17,18-EpETE occurred in the plasma, cerebral cortex, heart, kidney, liver, lung, pancreas and erythrocytes of rats fed a diet rich in EPA and DHA [30]. The first evidence that ω -3 PUFA epoxides have potent biological activity was the finding that EpDPEs inhibited AA-induced platelet aggregation more effectively than EETs [31].

4. Metabolism of PUFA epoxides

Most of the information about the metabolism of PUFA epoxides has been obtained with EETs. Fig. 3 shows the metabolic pathways using 14,15-EET, a widely studied EET, as an illustration. The major pathways are incorporation into phospholipids and hydrolysis by soluble epoxide hydrolase (sEH) which converts EETs to the corresponding dihydroxyeicosatrienoic acids (DHET) [1,8,9]. 14,15- and 11,12-EET also can be converted to the minor metabolites shown in Fig 3 under certain conditions [22],

EpETEs, EpDPEs and EpOMEs also are incorporated into phospholipids and hydrolyzed by sEH [1,16,26]. However, there is no information whether the minor metabolites are produced from these PUFA epoxides.

4.1. Uptake into cells and tissues

Unesterified EETs can be taken up from the extracellular fluid by many different types of cells [9]. It is not known whether a transporter is required for EETs to cross the plasma membrane, but cytosolic fatty acid binding proteins (FABPs) bind EETs and may enhance uptake by facilitating EET desorption from the membrane [32]. The K_d for EET binding to heart-FABP ranges from 0.4 to 1.7 μ M, which is 7- to 30-times higher than the K_d for AA binding. Yet, binding to FABP is sufficient to protect EETs from hydrolysis by sEH, and modeling studies suggest that binding increases the intracellular retention of unesterified EETs and prolongs their functional effects [33].

EETs, ω -3 PUFA epoxides, and the corresponding diols are present in the phospholipids and triglycerides of plasma lipoproteins [1,26]. These compounds are released when very low-density lipoproteins are hydrolyzed by lipoprotein lipase, and they represent another extracellular source of preformed PUFA epoxides for uptake by the tissues [34].

4.2. Incorporation into phospholipids

EETs are incorporated rapidly into the *sn*-2 position of cell phospholipids through a coenzyme A-dependent mechanism [1,9]. Studies with an insulinoma cell line suggest the acyl-CoA synthetase 4 isoform may mediate the formation of the EET-CoA intermediate required for the esterification reaction [35]. The largest amounts of EETs are present in phosphatidylcholine, but a relatively high percentage of 14,15-EET is incorporated into phosphatidylinositol [9].

The functional role of EET incorporation into cell phospholipids is still uncertain. The most likely possibility is that phospholipids provide a temporary storage site for excess EETs until they can be hydrolyzed and cleared from the cell. However, cardiac L-type Ca^{2+} channels are inhibited when they are reconstituted with phosphatidylcholine containing 11,12-EET at *sn*-2, suggesting that phospholipids containing EETs can modulate the function of membrane proteins [36]. Although EETs are estimated to comprise less than 0.01% of the total fatty acyl chains in cell phospholipids, this may be enough to affect localized membrane properties if the EETs accumulate in microdomains. Furthermore, EETs contained in phospholipids are rapidly released when endothelial cells are activated, suggesting that storage in phospholipids may provide an immediate source of EETs for the initiation of a functional response [9]. Phospholipids containing EETs also may be substrates for the synthesis of 2-epoxyeicosatrienoylglycerols, 2-arachidonoylglycerol analogues produced by kidney and spleen. These 2-epoxyeicosatrienoylglycerols bind to cannabinoid CB1 and CB2 receptors and have mitogenic activity [37,38].

4.3. Hydrolysis by soluble epoxide hydrolase

The PUFA epoxides are hydrolyzed to the corresponding diols by sEH, the member of the α/β hydrolase fold enzyme family [39]. In humans, sEH is encoded in *EPXH-2*, a single copy gene on chromosome 8. The enzyme is widely distributed in human tissues, with the largest amounts in liver, kidney, intestine, and the vasculature. sEH also is present in brain, and while it is expressed in some neurons, it is contained mostly in cortical and hippocampal astrocytes [40–42]. sEH functions as a 120 kDa homodimer, and dimerization is required for enzymatic activity [43]. Each subunit contains a N-terminal phosphatase domain and C-terminal hydrolase domain, and these domains function independently [44,45]. The phosphatase domain hydrolyzes isoprenoid phosphates and lysophosphatidic acid [46,47], which are phosphorylated lipids that stimulate cell growth [48].

Although sEH hydrolyzes all of the commonly occurring PUFA epoxides, human sEH has the highest catalytic efficiencies with 9,10-EpOME, 14,15-EET and 13,14-EpDPE [16]. The enzyme exhibits selectivity for the predominant enantiomeric forms present in tissues [49]. Hydrolysis begins rapidly when cells take up EETs, and some conversion of 14,15-EET to 14,15-DHET in vascular smooth muscle cells has been observed during the first 3 min of

incubation [9]. Although DHETs are released into the extracellular fluid, endothelial cells can incorporate small amounts of DHETs into phosphatidylcholine and phosphatidylinositol [50]. DHETs and diols formed from EpETEs and EpDPEs also are present in rodent brain and spinal cord [16,51], as well as in the plasma of humans and piglets fed ω -3 PUFA supplements [26,52].

Other members of the α/β hydrolase fold enzyme family that hydrolyze PUFA epoxides include microsomal epoxide hydrolase (mEH) and ABHD9 (EH3) [53]. Although mEH and sEH have complementary and partially overlapping substrate selectivity, the most widely accepted role of mEH is in xenobiotic metabolism. sEH will dominate hydration of most fatty acid epoxides, except possibly in regions of the brain where mEH protein levels are relatively high. The expression of EH3 appears to be under careful regulation and is high in lung, skin and upper gastrointestinal tract [53]. This suggests a possibly important functional role of EH3, but further confirmation is required. The sEH inhibitor 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) has been reported to inhibit EH3 [53], but the newer sEH inhibitors that contain bulky substituents on both sides of the urea group do not inhibit EH₃.

4.3.1. sEH inhibition—The term sEH inhibitor as commonly used refers only to inhibition of the sEH hydrolase activity. sEH inhibition stabilize EETs, increases their incorporation into phospholipids and other metabolites, and enhances their functional responses [54], effects that have potential therapeutic benefits [55–57]. Recent findings indicate that sEH inhibitors have similar stabilizing effects on ω -3 PUFA epoxides [16,51]. Although inhibitors of the sEH phosphatase activity are available [58,59], few studies have been done with them because the physiological importance of the sEH phosphatase activity is uncertain.

4.3.1.1. Inhibitors and analogues used to study PUFA epoxide function: Selective sEH inhibitors are the most commonly used compounds to study the functional effects of PUFA epoxides [14,16,55,]. They include the potent *N,N'*-disubstituted urea inhibitors like AUDA, and those with higher water solubility such as *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB), and 1-(1-methylsulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)urea (TUPS) [60]. Dual function sEH inhibitors are available now, including those that also serve as stable EET analogues such as 8-HUDE (12-(3-hexylureido) dodec-8-enoic acid) [61,62], those that modulate peroxisome proliferator-activated receptors [63], and those that inhibit 5-lipoxygenase [64]. In addition, inhibitors with new pharmacophores are being developed [65–67], including urea-containing-pyrazoles that are dual inhibitors of sEH and cyclooxygenase-2 [68]. Furthermore, in addition to inhibiting sEH, *N*-cyclohexyl-*N'*-dodecanoic acid urea (CUDA) and AUDA are weak activators of peroxisome proliferator-activated receptor (PPAR) α [69].

Other compounds used to study PUFA epoxide function include *N*-methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH), a selective inhibitor of CYP epoxygenase [70], and 14,15-epoxyeicosa-5(*Z*)-enoic acid (14,15-EEZE), an EET antagonist [71]. Compounds containing epoxide bioisosteres that have anti-arrhythmic effects similar to 17,18-EpETE on the heart [61,72], and selective inhibitors of the vascular actions of 14,15- and 11,12-EET also are available [73,74].

4.3.2. Function of PUFA diols—DHETs and the diol metabolites of EpETE and EpDPE have little or no effect on many of the physiological functions mediated by the corresponding PUFA epoxides [16,18,30,54]. Furthermore, no phenotype was observed in *EPXH2* gene deleted mice [75]. PUFA diols are much more polar than the corresponding PUFA epoxides. They accumulate primarily in the extracellular fluid and are generally thought to be inactivation products that move rapidly away from their sites of production and are excreted. However, some PUFA diols have biological activity. DHETs augment bradykinin-induced relaxation of porcine coronary artery rings [19], dilate canine and human coronary arterioles [76,77], and activate BK_{Ca} channels [78]. 14,15-DHET activates PPAR α -mediated gene expression [79,80], 11,12-DHET regulates cAMP production in HEK293 cells [81], and PUFA diols including 12,13-DHOME are required for optimal progenitor cell proliferation and vascular repair [82]. Diol metabolites produced by sEH are essential for monocyte chemotaxis to monocyte chemoattractant protein-1 [83]. These findings indicate that some PUFA diols may have functional effects on physiological processes.

4.4. Additional metabolic pathways

Additional EET metabolites are produced under certain conditions. 11,12- and 14–15-EET undergo partial β -oxidation to a 16-carbon metabolites and chain-elongation to a 22-carbon metabolites that have bioactivity [22,84,85]. Likewise, 11,12-DHET undergoes partial β -oxidation to a 16-carbon metabolite in porcine aortic smooth muscle cells [9]. However, appreciable amounts of these products are formed only when sEH is deficient or inhibited [86,87], suggesting that these metabolic pathways probably have a minimal role under ordinary physiological conditions. EETs also can undergo ω oxidation, and the ω -hydroxy-EET products activate PPAR α [88]. In addition, the perfused rabbit kidney converts 5,6-EET to the prostaglandin derivative 5,6-epoxy-PGE₁ which produces renal vasodilation [89].

Human liver CYP3A4 and brain CYP2B6 and CYP2D6 oxidize arachidonoyl ethanolamide (anandamide) to EET ethanolamides [90,91]. 5,6-EET-ethanolamide activates the CB2 cannabinoid receptor, and 14,15-EET-ethanolamide is a weak activator of the CB1 cannabinoid receptor [91,92]. The EET-ethanolamide regioisomers can be ω -hydroxylated or hydrolyzed to the corresponding diol derivatives by sEH or microsomal epoxide hydrolase [92]. DHA-ethanolamide which has synaptogenic and neurogenic properties is synthesized in the brain [93,94], but there is no information regarding whether it can be epoxidized to EpDPE-ethanolamides.

5. Mechanism of PUFA epoxide action

There is increasing evidence that many EETs function through a membrane receptor mechanism as illustrated schematically by the solid arrows in Fig. 4. EET-dependent vasodilation occurs through the cAMP/protein kinase A (PKA) signaling pathway activated by a receptor coupled to G_{as}-protein [96,97], and other EET-dependent functions are mediated by activation of PI3K/Akt, MAP kinase or Src kinase pathways [22]. EpETEs and EpDPEs affect many of the same processes, and thus, it is likely that they act through a similar receptor-dependent mechanism. However, EETs also enter cells and can interact

directly with intracellular effectors like FABPs and PPAR γ [20,32], cardiac K_{ATP} channels [98], and TRPV4 Ca²⁺ channels [99].

5.1. Putative EET-selective membrane receptor

Recent results support the probability that 14,15-EET acts by binding to a membrane receptor that activates signal transduction pathways [100]. 14,15-EET-complexes that do not enter cells produced relaxation of coronary artery rings and inhibited cAMP-induced aromatase activity with the same potency as 14,15-EET in solution [101,102]. 11,12-EET activated the vascular BK_{Ca} channel through ADP-ribosylation of the G_{αs} protein, providing evidence for a G-protein coupled EET receptor [96]. Furthermore, 20-iodo-14,15-epoxyeicosa-8(Z)-enoyl-3-azidophenyl-sulfonamide, a photoaffinity probe that relaxes bovine coronary arteries, labeled a 47kDa protein in bovine coronary artery and U937, endothelial and vascular smooth muscle cells. Labeling was inhibited by low concentrations of 11,12- and 14,15-EET, indicating that the 47 kDa protein may be the putative high-affinity EET receptor [103].

The results provide substantial evidence for a receptor-dependent mechanism of EET action, but some caution is indicated because the putative receptor has not been cloned, and the sulfonamide photoaffinity probe did not label transfected HEK293 cells that expressed 79 G-protein coupled receptors [103]. Moreover, it seems unlikely that a single receptor mediates all of the diverse functions of EETs [22]. In this regard, some EET responses involve receptors for other lipid mediators. For example, 14,15-EET produced vasodilation of rat mesenteric artery by activation of the prostaglandin EP₂ receptor [104]. 14,15-EET also relaxed the aorta and mesenteric resistance arteries by acting as an antagonist of the thromboxane receptor [105]. Weak binding of EETs to several receptors that mediate nervous system signaling also has been observed, including the peripheral benzodiazepine, cannabinoid CB₂, neurokinin NK₂ and dopamine D₃ receptors [106].

5.2. Modulation of ion transport

EET-dependent activation of BK_{Ca} channels produces vasodilation through hyperpolarization of the vascular smooth muscle [21]. Like EETs, EpDPEs and EpETEs are potent activators of BK_{Ca} channels. 13,14-EpDPE activates rat coronary smooth muscle BK_{Ca} channels and dilates coronary microvessels with the same efficacy as 11,12-EET [107,108], and it is 1000-times more potent than EETs in activating the BK_{Ca} channels of porcine coronary myocytes and rat small coronary arterioles [109]. 16,17-EpDPE also activates BK_{Ca} channels [108]. 17,18-EpETE produces relaxation of human pulmonary artery tissue by activating BK_{Ca} channels [110], and BK_{Ca} channels activation by 17(R), 18(S)-EpETE in cerebral arteries occurs by targeting the channel α -subunit [111,112].

EETs stimulate an influx of Ca²⁺ by activating transient receptor potential (TRP) channels [5]. TRPV4 channels are activated by 5,6-, 8,9- and 11,12-EET [99,113,114], possibly through a direct effect of the EET on the TRPV4 channel [7]. 11,12-EET activated TRPC6 channels by increasing their translocation to the plasma membrane [115,116], and 8-HUDE, the sEH inhibitor that also functions as an EET agonist, increased Ca²⁺ influx into pulmonary artery smooth muscle cells by increasing the expression of TRPC6 and TRPC1

channels [62]. In addition, EETs increased Ca^{2+} influx into vascular smooth muscle cells by activating voltage-dependent Ca^{2+} channels [117].

EETs inhibit Na^+ channels by decreasing their open probability [118,119]. Inhibition of the epithelial Na^+ (ENaC) channel is due to an ERK1/2-dependent phosphorylation of the ENaC β - and γ subunits [120]. Down-regulation of the ENaC channel also occurred when endogenous EpETEs and EpDPEs were increased in mice by a combination of an ω -3 PUFA diet was combined with a sEH inhibitor [121]. 11,12-EET directly activates the cardiac K_{ATP} channel by reducing its sensitivity to ATP [96], but it activates mesenteric artery K_{ATP} channels through the receptor-dependent PKA pathway [122]. EpDPE regioisomers are more potent activators of K_{ATP} channels than the corresponding EETs [24]. EETs inhibit the volume-activated chloride channels (VACC) in rat mesenteric arterial smooth muscle cells through a cGMP-dependent mechanism [123].

6. Physiological functions of PUFA epoxides

The effects of EETs on vascular reactivity, renal function and blood pressure regulation have been reviewed in detail [3–5,7,55,56]. This section focuses primarily on other actions of EETs and the effects of ω -3 PUFA epoxides on these processes.

6.1. Angiogenesis

Stimulation of endothelial cell proliferation, migration and tube formation occurred in cerebral microvessel endothelial cells co-cultured with astrocytes which release EETs into the medium, or when incubated in a medium containing 8,9-EET [124,125]. Similar angiogenic effects occurred when CYP2C9 was overexpressed in human lung microvascular endothelial cells or when these cells were incubated with 14,15-EET [126]. 11,12-EET-induced angiogenesis in human umbilical vein endothelial cells occurred through PI3K/Akt mediated phosphorylation of FOXO factors which caused down-regulation of the cyclin dependent kinase inhibitor p27^{Kip1} [127]. Other mechanisms that have been observed in EET-stimulated angiogenesis include activation of matrix metalloproteinases, MAP kinase, ERK, JNK/cJun, sphingosine kinase-1, EphB4-coupled to PI3K/Akt, and Src kinase coupled to PI3K/Akt or STAT-3 phosphorylation [128–135]. The angiogenic effect of 11,12- and 14,15-EET in a mouse wound healing model occurred through up-regulation of vascular endothelial growth factor [136].

As opposed to EETs, ω -3 PUFA epoxides suppress angiogenesis. 19,20-EpDPE and other EpDPE regioisomers decreased vascular endothelial growth factor-induced angiogenesis in mice and suppressed fibroblast growth factor 2-induced migration and protease production in human umbilical vein endothelial cells [18]. 17,18-EpETE also decreased the proliferation of transformed brain microvessel endothelial cells through down-regulation of the cyclin D1/cyclin dependent kinase 4 complex mediated by the p38 MAK kinase pathway [137].

6.2. Tumor growth and metastasis

EETs and ω -3 PUFA epoxides also have opposite effects on tumor proliferation and metastasis [138]. EETs stimulated escape from tumor dormancy and multi-organ metastasis

in mouse tumor models [139], and they increased proliferation and decreased apoptosis of hematologic malignancies by activating AMP kinase, cJun, PI3K/Akt and EGF receptor phosphorylation [140]. 11,12-EET increased motility of prostate cancer cell lines by activating EGF receptor and PI3K pathways [141], and 14,15-EET stimulated the growth of estrogen receptor-positive breast cancer cell lines by promoting STAT-3 phosphorylation [142]. EETs also promote tissue and organ regeneration [143]. Conversely, ω -3 PUFA epoxides decrease tumor growth and metastasis [18]. 19,20-EpDPE combined with a low dose of the sEH inhibitor *t*-AUCB decreased the growth of the Met-1 breast cancer in mice and reduced metastasis of the Lewis lung carcinoma by 70%, and 16,17-EpDPE alone or combination with *t*-AUCB reduced Lewis lung carcinoma metastasis. The anti-metastatic effect of the EpDPEs occurred at the site of metastasis, possibly by blocking tumor angiogenesis [15].

6.3. Inflammation

EETs and ω -3 PUFA epoxides have anti-inflammatory effects in the cardiovascular system, bronchi, kidney and nervous system [16,144,145]. The vascular effect is due to attenuation of NF- κ B-dependent inflammatory responses [145]. 8,9-EET attenuated lipopolysaccharide-stimulated nuclear translocation of NF- κ B in mouse B lymphocytes and decreased basal and activation-induced antibody secretion [146]. 11,12- and 14,15-EET decreased oxidized low density lipoprotein-mediated inflammation in endothelial cells by inhibiting LOX-1 receptor up-regulation and NF- κ B activation [147]. TNF- α induced inflammation and apoptosis in rat cardiomyocytes occurred through a mechanism involving PPAR γ [148], and 17,18-EpETE attenuated the TNF α -stimulated inflammation in human bronchial explants by targeting PPAR γ and reversing the phosphorylation of p38 MAP kinase [149]. Anti-inflammatory effects of PUFA epoxides also occur in the kidney. An orally active stable EET analogue attenuated kidney injury in the Dahl salt-sensitive rat by decreasing oxidative stress, endoplasmic reticulum stress and inflammation [150]. Likewise, increases in EpETEs and EpDPEs produced by administration of a diet rich in ω -3 PUFAs combined with a sEH inhibitor decreased renal markers of inflammation in angiotensin-II-dependent hypertension [121].

Results obtained in mice fed a high-fat diet indicate that sEH deficiency or inhibition attenuates endoplasmic reticulum stress in the liver and adipose tissue. Furthermore, insulin signaling increased when HepG2 cells were incubated with EETs or EpOME [151]. Treatment of mice fed a high-fat diet with the sEH inhibitor TUPS also decreased insulin resistance and glucose concentration [152]. These findings suggest that PUFA epoxides may be physiological modulators of endoplasmic reticulum stress [151].

6.4. Nervous system function and pain

EETs modulate neurohormone and neuropeptide release, integrate cell-cell communication, produce neurogenic vasodilation, and protect against the effects of cerebral ischemia [153]. 14,15-EET stimulates axon growth in primary cortical and sensory neuron cultures [154], and EETs mediate cerebral vasodilation produced by stimulation of astrocyte metabotropic glutamate receptors, possibly by activating K⁺ channels or TRPV4 channels [114,155,156]. When injected into the rat brain, 14,15-EET produced an anti-nociceptive effect through

activation of β -endorphin and met-enkephalin [157], and it reduced the injurious effect of H_2O_2 in the dopaminergic N27 neuronal cell line [158]. The administration of sEH inhibitors delayed the onset of seizures mediated by GABA, indicating that EET stabilization can suppress pathologic neurotransmission in the brain [159].

EpDPEs are present in brain and spinal cord and have direct anti-hyperalgesic actions in a rat model of pain associated with inflammation [16]. Although 7,8-EpDPE accounts for 85–90 % of the total EpDPE in neural tissues, 13,14-EpDPE has the most potent anti-nociceptive effect. PUFA epoxide levels in the plasma and spinal cord, including EpDPEs and EpETEs, were stabilized by administration of sEH inhibitors, and this augmented their effectiveness in reducing neuropathic pain in a rat model of diabetes [51]. The anti-hyperalgesic effects of EETs result from repression of the *COX2* gene and rapid up-regulation of the acute neurosteroid-producing gene *StARD1* [160]. It is possible that these mechanisms also may mediate in the anti-nociceptive effect of EpDPEs [16].

6.5. Cardioprotective actions

EETs and the ω -3 PUFA epoxides have protective effects on the heart. Infarct size in the dog heart resulting from coronary occlusion was decreased by administration of 11,12- or 14,15-EET. The protective effect of these EETs was potentiated by the administration of a sEH inhibitor and abolished by the EET antagonist 14,15-EEZE [161]. sEH inhibition also produced cardioprotection by increasing endogenous EET levels in the dog heart [162]. Treatment of mice with sEH inhibitors after myocardial infarction reduced cardiac ischemia-reperfusion injury, cardiac remodeling and fibrosis [163,164]. Addition of 14,15-EET to rat cardiomyocytes decreased mitochondrial dysfunction due to cellular stress by a K^+ channel dependent mechanism [165], and treatment with 11,12-EET protected the rat heart following myocardial infarction through a mechanism involving activation of a $G_{i/o}$ protein coupled to δ - and μ -opioid receptors [166].

Consistent with these EET-dependent protective actions, overexpression of human CYP2J2 in mouse cardiomyocytes protected against doxorubicin-induced cardiotoxicity [167], and endothelial expression of CYP2J2 reduced infarct size and increased left ventricular developed pressure after ischemia-reperfusion [168]. By contrast, endothelial expression of CYP2C8 in mice impaired functional recovery of the heart due to increased production of reactive oxygen species. This caused oxidation of linoleic acid to 9,10-DiHOME which increased coronary resistance [169].

EpETEs and EpDPEs protect the heart because they have potent anti-arrhythmic actions [30]. A negative chronotropic response occurred almost immediately after 30 nM 19,20-EpDPE or 17,18-EpETE was added to rat cardiomyocytes exposed to high concentrations of Ca^{2+} . The effect of 17,18-EpETE was stereoselective and 17(*R*),18(*S*)-EpETE, the active enantiomer, was 1000-fold more potent than EPA [30]. The quantity of EpETEs and EpDPEs in left ventricular myocardium increased when rats were fed a high ω -3 PUFA diet, suggesting that the anti-arrhythmic effect of dietary ω -3 PUFAs is due to an increase in the amount of ω -3 PUFA epoxides in the myocardium [17]. Stable epoxide bioisosteric analogues that have a more potent negative chronotropic effect than 17,18-EpETE have been

synthesized and may serve as templates for the development of clinically useful anti-arrhythmic agents [72].

7. Conclusions and future directions

Recent findings in animal models indicate that the EETs synthesized from AA have potentially beneficial angiogenic, anti-inflammatory and cardioprotective effects in addition to their well-known actions on vascular and renal function. Likewise, ω -3 PUFA epoxides synthesized from EPA protect against cardiac arrhythmias, and those synthesized from DHA reduce inflammatory and neuropathic pain and prevent tumor metastasis. Translational studies are needed to determine whether these effects of PUFA epoxides are functionally important in humans. Additional studies also are needed to identify the one or more putative EET receptors and to determine whether all of the actions of PUFA epoxides are mediated by receptor-dependent signaling mechanisms. The potent selective sEH inhibitors and stable epoxide bioisosteric analogues now available provide new pharmacological approaches for the treatment of many serious diseases. Furthermore, the possibility of increasing the tissue levels of ω -3 PUFA epoxides to protect against cardiac arrhythmias and tumor metastasis offers a new paradigm for the treatment of heart disease and cancer. The challenge for translational research is to determine whether these pharmacological and dietary interventions targeted to PUFA epoxides actually will produce beneficial outcomes for human health.

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Highlights

- PUFA are oxidized to PUFA epoxides by cytochrome P450 epoxygenases
- PUFA epoxides function as mediators in the cardiovascular, renal and nervous system
- PUFA epoxides activate receptor-mediated signaling pathways and ion channels
- Soluble epoxide hydrolase inactivates PUFA epoxides by hydrolysis to PUFA diols
- Inhibition of soluble epoxide hydrolase potentiates the action of PUFA epoxides

Fig. 1.

EET synthesis in endothelial cells. In response to bradykinin or other vasodilators, AA contained in endothelial phospholipids is released by a Ca^{2+} -activated phospholipase A₂ (PLA₂). CYP epoxygenases oxidize the AA to EETs in a reaction that utilizes NADPH and O₂, and the EETs are released from the cell. Although CYP epoxygenases can synthesize all four EET regioisomers as illustrated in the figure, 11,12- and 14,15-EET are the most abundant EETs produced by the endothelium and many other tissues. DHA or EPA epoxidation by CYP epoxygenases has not been investigated in intact cells, so it is uncertain whether this receptor-mediated production mechanism also applies to ω -3 PUFA epoxides.

Fig. 2.

Epoxides derived from LA and ω -3 PUFAs. The most prominent regioisomers include 9,10-epoxyoctadecaenoic acid (9,10-EpOME) from LA, 17,18-epoxyeicosatetraenoic acid (17,18-EpETE) from EPA and 19,20-epoxydocosapentaenoic acid (19,20-EpDPE) from DHA. Although minor, 13,14-EpDPE is a potent activator of coronary BK_{Ca} channels and reduces inflammatory and neuropathic pain.

Fig. 3.

Pathways of cellular EET metabolism. The illustration is for 14,15-EET, but the other EET regioisomers also are metabolized by the major pathways indicated by thick arrows with the exception of 5,6-EET which is a poor substrate for sEH. Cytosolic FABP binds to EETs, which may enhance EET uptake, although requirement for a transporter has not been demonstrated. Following uptake, the EET is either incorporated into phospholipids or hydrolyzed by soluble epoxide hydrolase (sEH) to the corresponding dihydroxyeicosatrienoic acids (DHET) which is excreted to the extracellular matrix. The mechanism of DHET exit from the cell has not been determined. Incorporation of the EET into phospholipids is mediated by a membrane-bound lysophospholipid acyltransferase (LPAT) that requires ATP and CoA. The membrane-incorporated EET is released by a phospholipase (PLA₂) and can be either hydrolyzed by sEH or converted to minor metabolites. The minor metabolites include the partial β -oxidation product 10,11-Ep-16:2, chain elongation product 16,17-Ep-22:3 and ω -oxidation product 14,15-EET(20-OH). Appreciable amounts of these metabolites are produced only if sEH is deficient or inhibited, and some of the minor metabolites have been observed only in one cell type.

Fig. 4. Mechanisms of action of EETs. 11,12-EET is used in this illustration. Many EET functions occur through a membrane receptor-dependent mechanism which activates signal transduction pathways that modulate ion channels and transcription factors in the target cell. This mechanism is indicated by the solid arrows. Some EET responses may occur through intracellular effects or direct interactions with ion channels. Because the physiological relevance of these direct acting mechanisms is less substantial, they are illustrated by the dashed arrows. Listed are the signal transduction pathways, ion channels and transcription factors that are targeted by EETs in various cells, and the functional responses that occur in the cardiovascular, renal, and nervous system.