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Review

Epoxide hydrolases: their roles and interactions with lipid metabolism

John W. Newman, Christophe Morisseau, Bruce D. Hammock *

Department of Entomology, UCDavis Cancer Center, University of California, One Shields Avenue, Davis, CA 95616, USA

Abstract

The epoxide hydrolases (EHs) are enzymes present in all living organisms, which transform epoxide containing lipids by the addition of water. In plants and animals, many of these lipid substrates have potent biologically activities, such as host defenses, control of development, regulation of inflammation and blood pressure. Thus the EHs have important and diverse biological roles with profound effects on the physiological state of the host organisms. Currently, seven distinct epoxide hydrolase sub-types are recognized in higher organisms. These include the plant soluble EHs, the mammalian soluble epoxide hydrolase, the hepoxilin hydrolase, leukotriene A_4 hydrolase, the microsomal epoxide hydrolase, and the insect juvenile hormone epoxide hydrolase. While our understanding of these enzymes has progressed at different rates, here we discuss the current state of knowledge for each of these enzymes, along with a distillation of our current understanding of their endogenous roles. By reviewing the entire enzyme class together, both commonalities and discrepancies in our understanding are highlighted and important directions for future research pertaining to these enzymes are indicated.

* Corresponding author. Tel.: +1 530 752 7519; fax: +1 530 752 1537.
E-mail address: bdhammock@ucdavis.edu (B.D. Hammock).

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Nomenclature

ChEH cholesterol epoxide hydrolase
COX cyclooxygenase, prostaglandin G/H synthase
DHET dihydroxy eicosatrienoic acid
DHO dihydroxy octadecanoic acid
DHOME dihydroxy octadecenoic acid
EH epoxide hydrolase
EET epoxy eicosatrienoic acid
EpOME epoxy octadecenoic acid
FABP fatty acid binding protein
JH juvenile hormone
JHEH juvenile hormone epoxide hydrolase
HPETE hydroperoxy eicosatrienoic acid
LDLR low density lipoprotein receptor
LPL lipoprotein lipase
LTA_4 leukotriene A_4
LTB_4 leukotriene B_4
mEH microsomal epoxide hydrolase
NFκB nuclear factor kappa B
PDK pyruvate dehydrogenase kinase
PMN polymorphonuclear leukocytes
PPARα peroxisome proliferator activated receptor alpha
sEH soluble epoxide hydrolase
TCPO 3,3,3-trichloropropene-1,2-oxide
THETA trihydroxy eicosatrieneoic acid

Contents

1. Introduction

The oxidation of unsaturated lipids routinely yields epoxide-containing compounds, many of which have important biological functions in a broad array of organisms. Both enzymatic [1–4] and autooxidative [5,6] routes of lipid epoxide synthesis have been reported. The chemical reactivity and resulting toxicity of epoxide containing chemicals can vary widely depending on chemical structure [7,8].

Multiple enzymes, including epoxide hydrolases (EHs), have evolved to transform epoxides into compounds with decreased chemical reactivity, increased water solubility [9], and altered biological activity. EHs are ubiquitously found in nature. To date five EHs have been described in vertebrates: soluble EH (sEH), microsomal EH (mEH), cholesterol EH (ChEH), hepoxilin hydrolase

and leukotriene A_4 (LTA_4) hydrolase [9–11]. Soluble EH orthologs are also found in plants, with roles in epoxy lipid metabolism [12–14], while the juvenile hormone EH (JHEH) is an epoxy-lipid metabolizing enzyme in insects with homology to mEHs [15,16]. The sub-cellular localization and reported endogenous substrates of these EHs are shown in Table 1. Microbial EHs have been recently discussed [17] and will not be considered here.

While the soluble and microsomal EHs show structural characteristics suggesting derivation from a common ancestral gene, the LTA_4 hydrolase is distinct [11,18]. Neither the ChEH nor the hepoxilin hydrolase have been suitably characterized to evaluate structural relationship to the other EHs [11]. However, the failure of the ChEH to form a covalent substrate intermediate suggests that it is structurally unrelated to the microsomal and soluble EHs [19]. While having different biochemical properties [20], the overlapping substrate specificity and sub-cellular localization of the sEH and the hepoxilin hydrolase suggest that these two enzymes may serve complimentary roles. The unique nature and relative importance of these two enzymes can still be debated as cytosolic hepoxilin EH-like activity is routinely reported [21].

If we consider the chemical reactivity of the various substrates, we can hypothesize two independent forces driving the evolution of these enzymes; cytoprotection vs. cellular signaling. Early investigations of these enzymes focused on their cytoprotective roles associated with toxicosis. While the mEH has a clear role in protecting cells from metabolically generated arene oxides [22–25], examples of cytoprotection mediated through other EHs are rare and generally irrelevant to environmental exposures [26,27]. The identification of endogenous substrates of these enzymes [4,28–30], and our growing understanding of their signaling functions is shedding light on the physiological roles of various EHs.

This review will focus on the distribution, regulation, substrate/product profiles, and the endogenous role of these enzymes within a greater context of lipid metabolism. The biochemical mechanisms of action, as well as a more global description of substrates and inhibitors of these enzymes have been reviewed elsewhere [11,31,32].

Table 1
Epoxide hydrolase localization and lipid substrates

Enzyme	Sub-cellular localization	Lipid substrates	References
Plant soluble EH	Cytosol; glyoxysomes	Epoxy fatty; acids hydroxy, epoxy fatty acids	[35,44]
Mammalian soluble EH	Cytosol; peroxisomes[a]	Epoxy fatty acids; fatty acid phosphates	[78,81,113,115,117]
Hepoxilin EH	Cytosol; platelet membranes	Hydroxy, epoxy fatty acids	[20,126]
LTA_4 hydrolase	Cytosol	5(6)-epoxyeicosa-poly-enoic acids	[266]
Microsomal EH	ER plasma memebrane	Epoxy steroids; epoxy fatty acids	[33,335,389]
Cholesterol EH	ER	Cholesterol epoxides	[366,437]
Juvenile hormone EH	ER	Juvenile hormones; epoxy fatty acids	[452,453]

[a] A low level tight association of the sEH with microsomes also occurs suggesting that some of this enzyme may be localized to the endoplasmic reticulum (ER).

2. Soluble epoxide hydrolases

A number of EHs are found as soluble proteins within various cells. These include the "soluble EHs" from plants and animals, the hepoxilin hydrolase, and the zinc-metalloprotein leukotriene A_4 hydrolase. These enzymes are predominantly, but not completely localized in the cytosol. Each of these enzymes is responsible for the hydrolysis of aliphatic epoxy fatty acids. With the exception of the LTA_4 hydrolase, the products of these reactions are the corresponding vicinal diols, when the starting material is a simple epoxy fatty acid (Fig. 1).

2.1. The plant sEHs

The sEHs isolated from plants are roughly 35 kDa α/ß-hydrolase fold enzymes, which can occur as either monomeric or dimeric proteins [33]. These enzymes show structural homology to the bacterial haloalkane dehalogenase and the C-terminal domain of the mammalian sEH [34].

To date, sEHs have been reported from nine plants, soybean (*Glycine max*) [35], mouse eared cress (*Arabidopsis thaliana*) [36], potato (*Solanum tuberosum*) [37], common tobacco (*Nicotiana tabacum*) [38], oilseed rape (*Brassica napus*) [39], pineapple (*Ananas comosus*) [40], spurge (*Euphorbia lagascae*) [41], rice (*Oryza sativa*) [42], and rough lemon (*Citrus jambhiri*) [14]. To our knowledge, the rice, tobacco, and pineapple gene products have yet to be expressed. EH activity has been also characterized in the particulate fractions of spinach (*Spinacia olerecea*) and apple (*Malus pumila*) [43], and the soluble fraction of the castor bean (*Ricinus communis*) [44], vetch (*Vicia sativa*) [12], maize (*Zea mays*), wheat (*Triticum aedivum*), celery (*Apium graveolens*), tobacco (*N. tabacum*) and soybean (*Glycine max*) [33]. It is evident that plants contain multiple EH isoforms. At least three isoforms have been indicated in soybean, while unique constitutive and infection-induced forms have been reported in tobacco [33].

2.1.1. Tissue distribution and sub-cellular localization

The plant soluble EHs have been isolated from or localized in germinated seeds, seedlings, roots, fruit, tubers, and leaves [14,33,35,37,40,41]. The tissue distribution is quite variable from

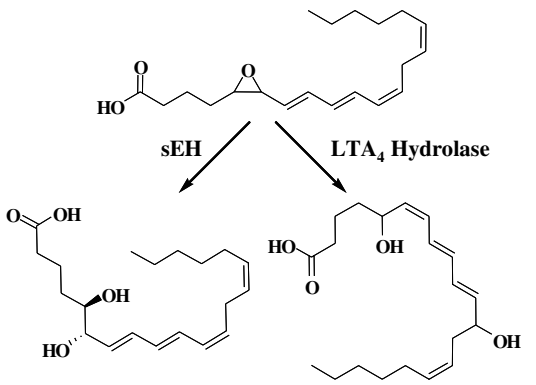


Fig. 1. Both LTA_4 hydrolase and the mammalian hepatic soluble EH can utilize leukotriene A_4 as a substrate. While the sEH produces a *vicinal threo*-diol from this substrate [121], LTA_4 hydrolase yields LTB_4 [323].

plant to plant, and underlines the overall lack of knowledge of the plant EHs. As with the mammalian soluble EHs, plant soluble EHs are found primarily in the cytosol, with a minor fraction being tightly associated with isolated microsomes [35]. In addition, subcellular fractionation of castor bean endosperm revealed a dual distribution of activity between the glyoxysomal and the cytosolic fractions [44], reminiscent of the dual distribution between peroxisomes and cytosol for the vertebrate orthologs described below.

2.1.2. Substrates

The plant sEHs characterized to date prefer *trans*- over *cis*-epoxides of sterically hindered substrates like stilbene oxides [39,45,46]. However, it appears that epoxide containing fatty acids are the preferred endogenous substrates of these enzymes. Plants produce an abundant array of epoxide containing lipids in biochemical cascades associated with host defense responses [47,48] and cutin polymer synthesis [13,49]. As shown in Fig. 2, these include epoxides of stearate and linoleate [50–52], as well as an array of epoxy, hydroxy lipids or hepoxilins (Fig. 2(d)) [53,54], and mid-chain epoxides with omega hydroxylations [49]. Evidence suggests that plant soluble EHs efficiently hydrolyze all of the compounds in Fig. 2 [39,45,46]. Both cress and potato EHs have also been shown to efficiently hydrolyze insect juvenile hormone, a tri-substituted epoxy terpenoid ester [46], suggesting that terpenic epoxides could be alternate or additional endogenous substrates for plant soluble EHs.

In relation to lipid metabolism, detailed biochemical investigations of the plant EHs have focused on their enantioselectivity. The most thoroughly studied enzyme in this class is the soybean EH, the first of the cloned and expressed EHs from a plant species [45]. The *G. max* EH has a strong enantio-preference for the 9(*R*),10(*S*)-epoxystearic acid ($E = 180 \pm 30$). The EHs characterized from potato, banana, and celeriac, as well as the constitutively expressed tobacco enzyme, also prefer the 9(*R*),10(*S*)-antipode, with *E*-values of 900 ± 200, 100 ± 30, 45 ± 15, and ~ 40,

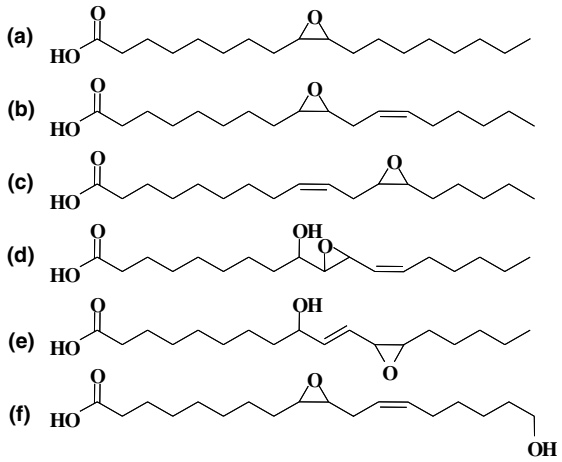


Fig. 2. Plants produce a diverse array of epoxide containing fatty acids substrates of the plant soluble EHs. These include: (a) 9(10)-epoxy octadecanoic acid; (b) 9(10)-epoxy octadeca-(12*Z*)-eneoic acid; (c) 12(13)-epoxy octadeca-(9*Z*)-eneoic acid; (d) 9-hydroxy-10(11)-epoxy octadeca-(12*Z*)-eneoic acid; (e) 9-hydroxy-12(13)-epoxy-octadeca-(11*E*)-eneoic acid, (f) 9(10)-epoxy-18-hydroxy octadeca-(12*Z*)-eneoic acid.

respectively [33]. The wheat, maize, rice, and infection-induced tobacco enzymes show little to no enantioselectivity ($1 \leqslant E \leqslant 4$). It has also been demonstrated that that the soybean, potato, and tobacco EHs stereo convert (±)9,10-epoxystearic acid antipodes by attack at the (*S*)-carbon to the corresponding *threo*-(*R,R*)-diol in >85% excess [33,35].

2.1.3. Regulation

While plants contain constitutive soluble EHs, inducible isoforms of these enzymes have also been reported [36,37,55]. For instance, the natural growth and differentiation of meristematic tissue is associated with increased EH transcription, in the potato leaf relative to the expanding and mature leaf [37]. Similarly, in the spurge (*E. lagascae*), a germination-specific EH has recently been reported [55]. The transcription of these inducible enzymes can also be increased by exogenous exposure to hormones involved in germination, development, growth, fruit ripening, and host-defense [36,37]. In particular, responsiveness to the growth hormones auxin and ethylene [36,56] and the host-defense regulator methyl jasmonate [37,57] have been noted. It is of equal interest to note that plant soluble EHs are not responsive to cytokinin, absisic acid, 6-benzylaminopurine, or gibberellin [36,37]. The interested reader is directed toward the following recent reviews for background on these hormones and their interactions [58–65].

In cress, the sEH transcript of the stems and leaves was weakly induced by drought stress, while auxin (indole acetic acid) and auxin mimics (e.g. 2,4-dichlorophenoxy acetic acid and naphthalene acetic acid) strongly induced this enzyme in pre-bolting young plants [36]. However, the EH activity level in vetch seedlings was insensitive to auxin mimics [12]. In the soybean, the sEH mRNA isolated from both germinating seeds and constitutive expression in the plant body showed induction by ethylene treatment [66]. In the potato, physical trauma of the leaf induced a sEH, as did exposure to exogenous methyl jasmonate [37]. Viral infection of the common tobacco has also been reported to increase the expression of sEH in aerial bodies of the plant [33,38]. Each of these examples therefore suggests that in plants soluble EHs are expressed in response to stress.

2.1.4. Physiological role: cutin biosynthesis and host defense

The substrate specificity and regulatory behavior of the plant soluble EHs argue for a primary function of this enzyme in host defense and growth. The defensive functions of these enzymes can be related to both passive (cutin biosynthesis) and active (anti-fungal chemical synthesis) roles. Cutin biosynthesis is also activated during initial plant growth and this may explain the association of heightened EH gene transcription during vegetative expansion.

Cutin is the waxy cuticle covering the aerial surfaces of plant providing a physical barrier to pathogens while allowing gas exchange [67]. The 9(10)-epoxy 18-hydroxy and 9,10,18-trihydroxy octadecanoic acids are common monomers of cutin poly esters in plants [13]. The enzymatic hydration of the 18-hydroxy-epoxystearic acid has been demonstrated in apples (*M. pumila*) [43]. It is of interest that these cutin monomers themselves are also messengers in plant–pathogen interactions that are released by fungal cutinases [68]. Consistent with an anti-fungal role, EH was induced in lemon leaves only after exposure to pathogenic fungus strains [14]. In addition, potato leaves efficiently synthesize the linoleate derived triols 9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*)-octadecenoic acid and 9(*S*),12(*S*),13(*S*)-trihydroxy-10(*E*)-octadecenoic acid, which have potent anti-fungal properties [53]. The enzymatic production of such substances has also been observed in garlic roots [54] and apple fruit [69]. Plants have also derived biosynthetic routes to prevent epoxy

fatty acid hydrolysis by sEHs. In particular, the in vivo synthesis of the linoleate 9,10-epoxide, or vernolic acid, appears to occur from linolyl-phosphotidyl choline, and the product is moved directly into triglycerides [52,70]. This route of synthesis thereby avoids interaction with sEHs, allowing epoxide accumulation in these seeds that are released upon germination [55].

It has also been reported that the (±)12(13)- but not (±)9(10)-epoxide of linoleic acid is a potent competitive inhibitor of allene oxide cyclase [71,72], a critical enzyme in jasmonic acid synthesis. While the physiological relevance of this observation has not been fully evaluated, it is intriguing that both allene oxide synthase [73] and at least one sEH [66] are ethylene inducible genes. Therefore, it is possible that the sEH also serves a role in regulating jasmonate signaling during periods of host response to attack by pathogens or insects.

2.2. The mammalian sEHs

The mammalian soluble EHs are homodimers, of ~62 kDa monomeric subunits [74] with isoelectric points between 5 and 6 [46,75]. Each monomer is comprised of two distinct structural domains, linked by a proline-rich peptide segment [34,76]. The epoxide hydrolase activity resides in the ~35-kDa C-terminal domain, which contains an α/ß-hydrolase fold structure homologous to the bacterial haloalkane dehalogenase, the plant soluble EHs and the microsomal EH [34]. The roughly 25-kDa N-terminal domain contains a distinct α/ß fold topology belonging to the haloacid dehalogenase enzyme superfamily [34,76]. The N-terminal domain catalytic site is a functional phosphatase [77,78], with apparent specificity for fatty acid diol phosphates [78]. In addition, the N-terminal domain appears to serve a critical role in stabilization of the domain-swapped architecture of the dimer [76].

Soluble EH activity has been documented in all vertebrates investigated including teleost fish: rainbow trout (*Salmo gairdneri)*, golden medaka (*Oryzias latipes*), fathead minnow (*Pimphales promelas*), marine scup (*Stenotomus chrysops)* [27,79,80]; rodents: mouse (*Mus mus*), rat (*Ratus norwiegicus*), rabbit (*Oryctolagus cuniculus*), guinea pig (*Cavia porcellus*), hamster (*Mesocricetus* sp.) [81–84]; domestic pig (*Sus domesticus*) [85]; domestic horse (*Equus caballus*) [86]; and primates: rhesus monkey (*Macaca mulatto*), baboon (*Papio* sp.), human (*Homo sapiens*) [74,87]. To our knowledge, the sEH has been cloned and expressed from the human [74], mouse [88], rat [89], and pig [85]. Based on analyses of the transcript sequences of the sEH genes of various organisms the enzyme is highly conserved [34].

2.2.1. Tissue distribution and sub-cellular localization

The sEH is broadly distributed in vertebrate tissues [10,90]. In mammals, activity has been detected in the liver [91], kidney [92,93], lungs [94,95], heart, brain, spleen [96], adrenals [97], intestine, urinary bladder [97], vascular endothelium and smooth muscle [93,98], placenta [99], skin [100], mammary gland [101], testis [96,102] and leukocytes [103]. The specific activity of sEH is highest in the liver, followed by the kidney, with lower levels in extra hepatic tissues [96,97]. The expression of sEH has also been observed in striated muscle [104] and ovary [105]. Immunoreactive proteins have also been reported in stomach, pancreas, prostate, tonsils, lymph nodes, and uterus [90]. While distribution of the sEH is diffuse in the liver [96], a more focal distribution is described for other tissues, and it appears to co-localize with cytochrome P450 2C9 in many tissues [90]. In the kidneys, the sEH appears concentrated in the renal cortex [106], and more spe-

cifically to the renal microvasculature [93] and possibly proximal tubule [90]. Similarly, sEH appears localized to vascular tissues in the lung [95]. The distribution of the sEH in glandular tissues appears complex, being localized to the adrenal cortex and peripheral islet cells in the pancreas, but diffuse in the pituitary [90].

Historically, the sEH was referred to as the cytosolic EH based on the primary isolation of characteristic activity in cytosolic cellular fractions [91,107]. However, sEH activity is also isolated in microsomal fractions. Early studies reported "an integral microsomal protein which is not dissociated from the membrane by repeated washing, high ionic strength salt, or chaotropic agent solutions, or by sonication" [108]. Later studies using both activity and immunological techniques have replicated this finding [106,109,110]. Therefore epoxide hydrolase activity observed in microsomal preparations should not be assigned to a specific hydrolase without conducting appropriate inhibitor or immunoprecipitation experiments. Besides the apparent microsomal association, the sEH has also been shown to localize in peroxisomes, being isolated in the light mitochondrial fraction [111]. Approximately 60% of the total sEH activity was isolated in the cytosol, and induction by clofibrate did not affect this distribution, while shifting cytosolic catalase activity from ~4% to 15–35% [112]. This dual compartmentalization on the sEH between the cytosol and peroxisome was later supported by the identification of an impaired peroxisomal targeting sequence at the carboxy terminal of the rat sEH [113], which is conserved in all cloned mammalian sEHs.

2.2.2. C-terminal domain substrates: epoxy fatty acids

The catalytic site situated in the C-terminal domain of the sEH is responsible for its well defined epoxide hydrolase activity [76,114]. As described for the plant sEHs, the vertebrate sEHs prefer *trans*- over *cis*-epoxides of sterically hindered substrates like stilbene oxides [81]. However, both saturated [115,116] and unsaturated [117] *cis*-epoxy fatty acids are excellent sEH substrates. As with plants, animals produce a broad array of epoxide containing aliphatic lipids, which have roles in the regulation of vascular tone, inflammation and cell growth [4,118]. With respect to the vertebrate soluble EH, the mono and diepoxides of unsaturated fatty acids have been the most thoroughly studied. To date, hydroxy, epoxy lipids (i.e. hepoxilins) have not been evaluated as substrates for this enzyme, however, considering the homology between the vertebrate and plant sEHs [33,46], these compounds are likely substrates.

As shown in Table 2, detailed biochemical evaluations have been reported with fatty acid monoepoxides and either purified or recombinant EHs from rodents. The reported K_m for epoxy lipids with rodent sEHs range from ~3 to 40 μM with maximum velocities ranging from not detectable to 9 μmol product/min/mg of protein. From the compiled results in Table 2, it can be seen that the sEH has a preference for epoxides distal to the carboxyl terminal and that it hydrolyzes 5,6-epoxy fatty acids poorly. Furthermore, sEH preferentially hydrolyzes the epoxyeicosatrienoic acid (EET) enantiomers that are the dominant endogenous products [119,120]. The elimination of olefins by catalytic hydrogenation reduced hydrolysis rates of the arachidonate derived epoxides, as did methylation of the free acids [120]. The enzymatic addition of water to the 11,12-EET antipodes and 14(S),15(R)-EET were not regioselective, while the 14(R),15(S)-EET was selectively hydrated at C15 and both enantiomers of the 8,9-EET, but not its methyl ester, proceeded by hydrolysis at C9 [120]. Increasing the number of *cis*-olefins appears to increase the efficiency and enantioselectivity of catalysis [33,119,120], however either the presence of *trans*-olefins, conjugated olefins, or *trans*-epoxides appear to reduce the affinity of epoxy fatty

Table 2
Specific activity of rodent sEHs with various epoxy lipids

Substrate	Absolute conformation	K_m (μM)	V_{max} (μmol/min/mg)	V_{max}/K_m	References
14,15-EET[a]	14(*R*),15(*S*)	4	9.03	2.3	[120]
	(±)	–	4.53	–	[119]
	14(*S*),15(*R*)	5	1.36	0.27	[120]
11,12-EET[a]	11(*S*),12(*R*)	4	3.02	0.76	[120]
	(±)	–	1.65	–	[119]
	11(*R*),12(*S*)	3	0.82	0.27	[120]
8,9-EET[a]	8(*S*),9(*R*)	5	3.10	0.62	[120]
	(±)	–	1.45	–	[119]
	8(*R*),9(*S*)	41	0.83	0.020	[120]
5,6-EET	(±)	–	<0.1	–	[119]
12,13-EpOME	(±)	6.2	2.67	0.43	[8]
9,10-EpOME	(±)	5.2	1.86	0.36	[8]
9,10-EpO	(±)	11	3.5	0.31	[116]
14,15-LTA_4[d]	(±)	11	0.90	0.081	[125]
14,15-LTA_4[b]	(±)	48	1.5	0.031	[84]
11,12-LTA_4[b,c]	(±)	18	2.4	0.13	[84]
5,6-LTA_4[b]	(±)	25	2.1	0.084	[84]
5,6-LTA_4	(±)	5	0.55	0.11	[265]

[a] Dominant endogenous antipodes.
[b] Purified guinea pig liver sEH; other reported values are for purified mouse sEH.
[c] 11(*S*),12(*S*)-*trans*-epoxy-(5*Z*,7*E*,9*E*,14*Z*)-eicosatetraenoic acid.
[d] 14(*S*),15(*S*)-*trans*-epoxy-(5*Z*,8*Z*,10*E*,12*E*)-eicosatetraenoic acid.

acids for the sEH. Regardless, the conjugated tetraeneoic fatty acid leukotriene A_4 is a substrate for the sEH purified from mouse liver, which produces the corresponding 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid [121]. This 5,6-diol is the predominant metabolite formed by LTA_4 hydrolysis in homogenates of kidney, heart, and brain [122]. The related 11,12- and 14,15-*trans*-epoxy tetraenoic fatty acids have also been reported as endogenous products of platelets [123] and HL-60 cells [124]. The formation of 14,15-dihydroxy eicosatetraenoic acid has been achieved in vitro using purified mouse soluble EH [125] and associated with pulmonary hepoxilin hydrolase activity [126].

In addition to the monoepoxy fatty acids, diepoxy fatty acids have also been reported as substrates for the sEH [127,128]. At a concentration of 7.5 μg/ml (i.e. ~1.2 M) affinity purified sEH transformed 9(10),12(13)-diepoxy octadecanoic acid into the corresponding tetraols, while a 20-fold dilution yielded only cyclization products containing dihydroxy tetrahydrofuran structures, without tetraol formation [128]. In vitro assays suggest that the sEH is responsible for the formation of these compounds in mammalian tissue homogenates [129], and these structures have been reported as mitogenic endocrine disrupting components in corn husks [130,131]. Fig. 3 displays two potential biosynthetic routes of tetrahydrofuran diol synthesis, both including an epoxide hydrolysis step. Regardless of the absolute route, tetrahydrofuran diols formation is dependent upon the oxidation of methylene interrupted olefins since larger cyclic products are

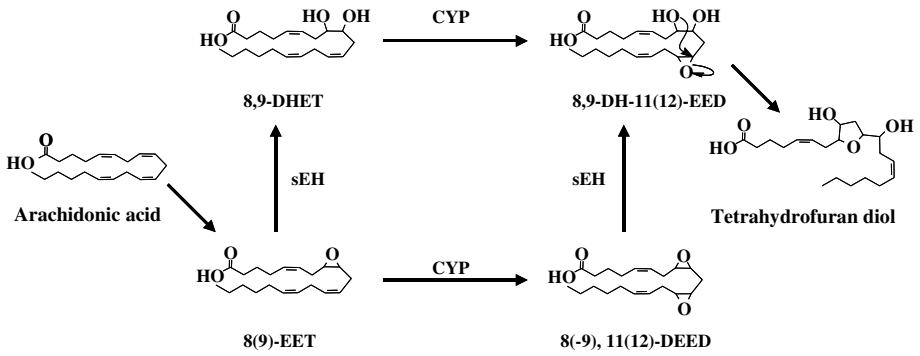


Fig. 3. Potential biological routes of terahydrofuran diol synthesis. The penultimate formation of a methylene-interrupted *vicinal*-diol epoxide, like 8,9-dihydroxy-11(12)-epoxy eicosadienoic acid (i.e. 8,9-DH-11(12)-EED), whether through diol epoxidation or diepoxides formation, leads to internal cyclization to form the furanyl lipid.

not observed [129]. Formation of trihydroxy furanyl lipids termed isofurans have also been reported in tissues under oxidant stress at high oxygen concentrations [132–134], being produced from hepoxilin like structures [134]. If an enzymatic route to the production of these compounds exists, it is unreported.

Finally, squalene-2,3-epoxide and diepoxide have been reported as substrates for the sEH [135]. The mono-epoxide is cyclized by lanosterol synthase during cholesterol biosynthesis. To our knowledge, the relevance of sEH in isoprenoid and sterol biosynthesis has not yet been reported.

2.2.3. N-terminal domain substrates: lipid phosphates

The catalytic site situated in the N-terminal domain of the sEH is responsible for the recently described phosphatase activity of this enzyme [77,78]. While the endogenous substrate of this enzyme has not been identified to date, current reports suggest that when identified, this will be a lipophilic phosphate [114], and possibly a phosphorylated lipid [78]. The crystal structure of the human enzyme revealed a "mitt" shaped N-terminal domain with a ~15 Å deep pocket containing the catalytic residues and magnesium binding site [114]. This catalytic site occurs along a 25 Å hydrophobic cleft that joins a ~14 Å tunnel lined with highly conserved residues, and the tunnel terminates near the interface of the N- and C-terminal domains [114]. The substrate specificity of this phosphatase site has been explored with a limited series of mono-phosphates of mono- and dihydroxy octadecanoids. The phosphorylation of *threo*-9,10-dihydroxy octadecanoic acid (*threo*-9,10-DHO; dihydroxy stearic acid) yielded the highest affinity substrate described to date ($K_m = 21$ μM, $V_{max} = 338$ nmol/min/mg) [78]. Furthermore, the *threo*-9,10-DHO mono-phosphates were hydrolyzed 3-times faster than the corresponding phosphorylated *erythro*-9,10-DHO (i.e. dihydroxy elaidic acid) [78]. Regioselectivity has also been suggested since incubation of a 1:1 mixture of the two hydroxy phosphate regioisomers of the *erythro*-9,10-DHO led to one of them, tentatively identified as the 10-phospho compound, being completely hydrolyzed before the other (Newman and Hammock – unpublished results). The presence of an olefin *beta* to the phosphate group increased activity, with *cis*-olefins potentiating substrate turnover 5-times greater than *trans*-olefins. It is possible that both the presence of the neighboring olefin and the carboxyl terminal are involved in orienting the substrate for the initial nucleophilic attack,

as found for epoxide hydrolysis by the C-terminal catalytic site [120]. It is intriguing that phosphorylation products of dihydroxy-fatty acids, endogenous products of the C-terminal domain active site, appear to be optimal substrates for the N-terminal domain of this enzyme. However it remains to be shown that this coincident substrate homology can be translated into a functional biochemical circuit in vivo, and that an enzymatic pathway exists to transform the product of one catalytic site to the substrate of the other. Regardless of the importance of dihydroxy fatty acid phosphates as endogenous substrates of this catalytic site, many other endogenous lipophilic phosphates have yet to be tested as potential substrate for this phosphatase activity, underlying that the knowledge of this activity is still in its infancy.

2.2.4. Regulation

While constitutively expressed, the vertebrate soluble EH is an inducible gene product, suggesting the need for regulation of this activity to compensate for changes in the internal chemical environment. For instance, the smoking of cigarettes has been shown to transiently reduce sEH activity, with the number of cigarettes smoked correlating with the decrease in activity [136]. More thoroughly studied is the pharmacological induction of sEH by exposure to peroxisome proliferator activated receptor alpha (PPARα) agonists like clofibrate, tiadenol or acetylsalicylic acid [96,137,138]. Most, but not all organisms appear to respond to these agents with a modest (2–3-fold) increase in hepatic sEH activity [139]. It is interesting that this PPARα induction also appears ineffective in evaluated extra-hepatic tissues [101,102,110], however this may be due to the rapid uptake and retention of these agents in the liver [140]. While PPARα response elements exist in the 5′-flanking region of the human sEH gene (*EPXH2*), whether or not these peroxisom proliferators response elements are functional is not known.

Therefore it can still be debated whether the peroxisome proliferator induction of the sEH is mediated through the direct interaction of PPARα ligands with the regulatory region of sEH or through secondary stimulation resulting from increased epoxy lipid formation concurrent with elevated lipid catabolism. For instance, fibrates [129] and free fatty acids [141] also induce microsomal cytochrome P450 epoxygenase activity, raising the possibility for substrate induction of sEH. This possibility has yet to be carefully evaluated. Consistent with induction of sEH by PPARα agonists, experimental diabetes and starvation also lead to a ~2-fold elevation sEH activity in the liver, along with a similar increase in beta-oxidation and a 3–6-fold increase in serum glucose [142]. The native sEH activity was restored by insulin administration [142]. In addition, the regulation of enzymes linked to gluconeogenesis (e.g. pyruvate dehydrogenase kinase; PDK) [143] and lipid oxidation (e.g. acetyl co-A synthetase) [144], show a similar pattern of regulation. Together theses results also suggest an unexplored link between sEH expression and the endogenous activity of the lipolytic enzyme, lipoprotein lipase (LPL) which releases endogenous PPARα ligands [145,146]. Interestingly, LPL expression also positively correlates with PPARα mRNA expression [147], and is suppressed by insulin [148], but is inhibited by PPARα ligands [149]. Finally, the PPARα-dependent induction of hypertension and diabetes by dexamethazone [150] suggests that evaluating the effect of dexamethasone in combination with PPARα agonists on sEH expression could be enlightening. Inspection of the 5′-flanking region of the *EPXH2* gene indicates the presence of glucocorticoid receptor response elements. Furthermore, it is possible that the decreased levels of insulin and increased levels of fatty acids and glucocorticoids associ-

ated with starvation and diabetes may be the mechanism behind sEH induction in these physiological states, as hypothesized for PDK [143].

A number of studies have also indicated hormonal regulation of the sEH in mammals, with sEH activity being elevated in males vs. females for both mice and rats [138,151–153]. In mice, the sexual dimorphism of sEH activity was more pronounced in the male kidney (283%) vs. the liver (55%), when compared to females [138]. Castration decreased activity in both organs, which was restored by testosterone supplementation [138]. Consistent with these observations, sEH gene transcription was also found to be induced by androgens in a castration/testosterone supplementation study of male rats [154]. In the later study, the drop in sEH occurred along with a set of oxidative stress-related genes, which included thioredoxin, peroxiredoxin 5, superoxide dismutase 2, glutathione peroxidase 1, microsomal glutathione-S-transferase, and glutathione reductase [154]. As in the castrated males, testosterone administration to females led to a more dramatic increase in kidney sEH activity than that of liver, while having no effect on unaltered males [138]. On the other hand, ovariectomy resulted in a 30% increase in sEH activity in both the liver and kidney of female mice [138]. In contrast, estradiol administration reduced hepatic sEH activity in males, while having no effect on intact females [153]. Interestingly, hypophysectomy (i.e. pituitary gland removal) lead to an increase in female hepatic sEH activity, while decreasing this activity in males [152] suggesting that these effects were due to the loss of gonadotropic hormones. Consistent with this supposition was the finding that growth hormone supplementation had no effect on sEH activity [152]. Therefore, it would appear that systemic sEH expression is under the control of the hypothalamic–pituitary–gonadal axis.

Developmental processes also regulate the levels of the sEH. Little is known about the importance of sEH in development; however the viability of sEH knockout mice [155] suggests that the lack of the adult hepatic gene is not critical in fetal development. The earliest sEH activity documented in vertebrate development was in the golden medaka, *Oryzias latipes*, a teleost fish, at 2 days post fertilization [27] corresponding to the late blastula formation, before significant cellular differentiation. Activity associated with sEH has been reported as early as 14 weeks of gestation in man, appearing in multiple tissues [97] without noticeable changes in activity [97,156,157]. In male rats, hepatic sEH increased steadily post-partum until puberty [151], while this activity in the liver and lung of horses were unchanged between weaning and adulthood [86]. Age-dependent changes in sEH have also been reported in male C57/B6 mice, where activity increased until 15 months then decreased by 59% at 30 months [158]. It is possible that these changes are directly related to androgen-dependent regulation of the sEH expression in the rodent, and may translate directly to man, where reductions in androgen production also occur with age [159,160].

2.2.5. Physiological roles

While yet to be fully characterized, significant insights into the endogenous role of the sEH have been gained recently. These advances have resulted from considering the biological pathways regulated and mediated by sEH substrates, the generation of sEH null mice [155], the use of metabolically stable sEH inhibitors [161,162], and the analysis of sEH polymorphs [163].

It is clear that the sEH plays a critical role in regulatory cascades influenced by epoxide-containing lipids. The best studied of the endogenous sEH substrates are the EETs, and a thorough review of the metabolism and biochemical function of these epoxy lipids has been recently published [4]. At the systemic level, the EETs have significant roles in the regulation of vascular,

cardiac, pulmonary, and renal physiology [4,164], being potent regulators of smooth muscle tone [165–168], cell proliferation [169] and migration [170]. The mechanisms by which epoxide hydrolysis affects EET activity is complex. The EETs are hydrolyzed to their corresponding *vicinal* diols or DHETs. Notably, epoxide hydrolysis reduces the rate of oxylipid esterification into phospholipids and promotes their excretion from cells [171], suggesting that the sEH may reduce the active pool of EETs available for release by activated phospholipases. In addition, the diols are released from cells in culture [8]. It is generally believed that epoxide hydrolysis eliminates the biological activity of these lipids. However, the DHETs are also active in some systems, including vasodilatation [172–174], tissue plasminogene activator stimulation [175], and sodium channel activation [176], however potency is generally reduced by hydrolysis in investigated systems. It is possible that the DHETs may have a physiological role that is yet to be described.

2.2.5.1. Blood pressure. The identification of epoxy fatty acids as potent vasodilators [177] suggested a role for the sEH in blood pressure regulation [4]. This hypothesis was confirmed with sEH-null mice, for which the male systolic blood pressure was reduced to female levels [155], suggesting an androgen dependent role in basal blood pressure regulation. These results are consistent with the natural sexual dimorphism of sEH expression [138]. As expected, hepatic and renal microsomes in these animals showed elevated EET and reduced DHET formation, supporting the hypothesis that the lack of sEH results in the elevation of endogenous vasodilators. Whether these changes were due to altered systemic vascular tone or renal heamodynamics is unclear, and both may be possible.

It should be noted that the sEH is localized to the renal microvasculature in humans, consistent with a role in renal hemodynamic regulation [93]. Reports of elevated sEH activity in the kidney of spontaneously hypertensive rats [106] and expression after angiotensin infusions [161] further suggest a link between sEH and blood pressure control under pathophysiological states. In each of these studies, blood pressure was reduced by the administration of potent sEH inhibitors, arguing that the sEH exhibits pro-hypertensive actions in these model systems. Consistent with this interpretation was the finding that the 14(15)-EET reduced renin release in cortical slices stimulated by the beta adrenergic antagonist isoproterenol, but had no effect on basal renin release [178]. The EETs also modulate the renal sodium/potassium ATPase acting as second messengers for the natriuretic effects of dopamine, parathyroid hormone and angiotensin II [179]. Finally, the sEH may modulate cardiac function by hydrolyzing 8(9)-EET, which inhibits sodium channel activation [176]. Together these findings suggest that the sEH has a complex role in the regulation of blood pressure.

2.2.5.2. Inflammation. The literature also supports a role for the sEH in the regulation of inflammation. In vascular endothelial cells, the 11(12)-EET displays anti-inflammatory properties, disrupting nuclear factor kappa B (NFκB) signaling and inhibiting cytokine-induced expression of cellular adhesion molecules [180]. This activity was diminished in the corresponding DHET [180], suggesting a pro-inflammatory role for the sEH. The 11(12)-EET is also a potent inducer of the anti-thrombotic agent tissue-specific plasminogen activator [175]. Since inflammation is pro-thrombotic [181], these results suggest that the lipid epoxides and sEH may play complex roles in the regulation of inflammation and thrombosis. The 14(15)-EET has also been shown to competitively inhibit the production of the pro-inflammatory agent prostaglandin E_2, potenti-

ating platelet-derived growth factor induced cellular proliferation [182]. The 14(15)-EET was also found to stimulate prostaglandin G/H synthase 2 (i.e. COX-2) expression, an effect which was potentiated with the use of sEH inhibitors [183]. The epoxy octadecenoic acids (EpOMEs) are also transformed by sEH, producing toxic [184,185] and inflammatory [186] dihydroxy octadecenoic acids (DHOMEs). This pathway of linoleate metabolism has been implicated in pathophysiological conditions including circulatory shock, disseminated intravascular coagulation [187], late phase death in severe burns [188], and adult respiratory distress syndrome [95]. With regard to inflammatory signaling, the DHOMEs were found to induce NFκB and interleukin-6 in a dose-dependent manner in vascular endothelial cell cultures [186]. The EpOMEs produced this effect in the absence, but not the presence of the sEH inhibitor 1-cyclohexyl-3-dodecyl urea [186], suggesting the dihydroxy lipids are pro-inflammatory agents. Mechanistically, the DHOMEs have also been shown to disrupt mitochondrial function [189], eliciting the mitochondrial permeability transition and leading to cellular apoptosis [190]. Therefore, it would appear that the sEH may play a key role in the regulation of inflammatory responses, degrading the anti-inflammatory and anti-thrombotic EETs and producing the pro-inflammatory DHOMEs. If true, the sEH may present a novel and valuable therapeutic target for the control of inflammation. Consistent with this hypothesis, it was recently shown that the administration of sEH inhibitors to rats receiving angiotensin II infusions prevented the progressive renal damage associated with this model system [162]. Therefore, investigating the regulation of sEH under multiple inflammatory states should prove informative.

2.2.5.3. Lipid and carbohydrate metabolism. The sensitivity of sEH to PPARα agonists and the abundance of sEH in peroxisomes argues for a role for this enzyme in lipid catabolism [147], however this link has not been adequately explored. It has been reported that the association of EETs with fatty acid binding proteins (FABPs) protects these epoxides from sEH-mediated hydrolysis [191] and FABPs can also be up regulated by PPARα agonists [192]. Therefore FABPs, which have roles in long chain fatty acid oxidation [193], may also offer a mechanism to regulate sEH-dependent epoxide hydrolysis, as well as a means of delivering these PPARα receptor ligands to the nucleus [194]. Recent investigations of genetic polymorphisms in the *EPXH2* gene have also suggested functional links between sEH and both plasma cholesterol/triglyceride homeostasis [195] and vascular disease [196]. Familial hypercholesterolemia results from the inheritance of a defective hepatic low density lipoprotein receptor (LDLR) leading to reduced rates of reverse cholesterol transport and increased plasma cholesterol concentrations. The prevalence of an Arg287-Glu mutation in the *EPXH2* gene was elevated in the familial hypercholesterolemic individuals, where the most common allele in the general population, i.e. Arg287/Arg287, was not observed [196]. Co-occurrence of the LDLR mutation and the Arg287/Glu287 genotype was associated with elevated plasma cholesterol and triglycerides, while Glu287/Glu287 individuals had normal plasma triglycerides [195]. Therefore, the Arg287Glu mutation may have a protective effect in individuals with familial hypercholesterolemia, while this mutation had no effect in the absence of the LDLR mutation. In contrast, the Arg287Gln mutation has recently been associated with an increased risk of coronary artery calcification in African Americans, but not Caucasian Americans [196]. Biochemical investigations of sEH polymorphs have suggested that manipulation of Arg287, specifically the Arg287Gln mutation reduces both epoxide hydrolase [197] and phosphatase activity, reduces enzyme stability, and destabilizes homodimer formation [198]. Therefore,

these studies of sEH polymorphisms suggest that the sEH may play a complex role in the homeostatic regulation of known risk factors of cardiovascular disease.

The fatty acid epoxygenase pathways have also been implicated in the hormonal regulation of glucose and lipid metabolism [199], suggesting that the sEH may be important in these system as well. In cultured pancreatic islet cells the 8(9)-, 11(12)-, and 14(15)-EET were found to stimulate glucagon release, but not effect insulin secretion [200]. The discovery of epoxygenases in this tissue [201] supports an autocrine role for these EETs in the pancreas. In hepatocytes, the EETs also stimulate vasopressin-induced glycogenolysis [202]. Cortisol secretion by the adrenal gland was also stimulated by 14,15-EET [203], which would promote gluconeogenesis, decrease glucose utilization, and increase circulating fatty acids. Together, these reports suggest that the EETs are hyperglycemic/hypolipidemic factors, and by corollary, the sEH may play a hypoglycemic/hyperlipidemic role in normal metabolism. If true, the induction of sEH by PPARα agonists may represent a homeostatic response to these anti-hyperlipidemic agents.

2.2.5.4. Reproduction. The sEH may also play roles in gonadal tissues. In the testis, the sEH is present along with epoxide synthesizing enzymes, and roles in epididymal motility and sperm concentration have been speculated [102]. In leutinized granulosa cells of the human ovary, nanomolar concentrations of the 14,15-EET have been reported to induce estrogen secretions [204]. In the porcine ovary, sEH expression was also seen to peak at estrus during the hours preceding ovulation, with elevated activity being observed in the cells of the granulosa vs. theca [85]. In addition, a unique gonadal sEH transcript, *EPXH2B* (NCBI Accession #: AY098585; Hennebold, J.D. and Adashi, E.Y.) has been identified in the mouse ovary, in which the first 44 amino acids of the expressed protein would be altered, eliminating phosphatase activity.

2.2.5.5. Phosphatase. The recent discovery of a catalytically active phosphatase in the N-terminal domain of the sEH raises new questions about the endogenous role of this enzyme. To date, studies suggest that the substrate of this domain is hydrophobic, and possibly a lipid phosphate [78]. As with other related phosphotransferases, a critical DXDX(T/V) catalytic motif is situated within 15 amino acids of the N-terminal [77]. Therefore the gonadal *EPXH2B* isoform should retain epoxide hydrolase but lack phosphatase activity. A thorough investigation of the substrate specificity and inhibitor sensitivity of the phosphatase domain will inevitably enhance our understanding of the role of the sEH.

2.3. Hepoxilin epoxide hydrolase

Hepoxilins are hydroxy epoxy metabolites of polyunsaturated fatty acids derived by hydroperoxide rearrangement (Fig. 4) [205,206]. An epoxide hydrolase with an apparent substrate preference for hepoxilins was partially purified from a rat liver cytosol preparation, and found to have an isoelectric focusing point of 5.3–5.4 and a molecular mass of ~53 kDa using sodium dodecyl sulfate electrophoresis [20]. The mass, high substrate selectivity and inhibition by μM concentrations of trichloropropene oxide suggest that this enzyme is distinct from the sEH. A detailed and direct comparison of these two mammalian cytosolic hydrolases has yet to be performed. Further purification and/or cloning of the hepoxilin EH have not been reported. However, the formation

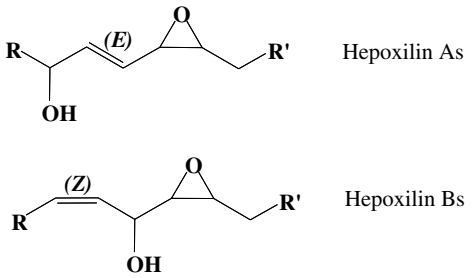


Fig. 4. Basic structure of the hepoxilin sub-families. Numerical subscripts indicate the number of olefins in the molecule such that those derived from arachidonic acid constitute the 3-series (A_3, B_3), while docosahexeneoic acid yields the 5-series (A_5, B_5) [223].

of trioxilins have been identified in various organisms including humans [126,207], rats [208], and the barnacles *Balanus amphitrite* and *Elminius modestus* [209].

2.3.1. Tissue distribution and sub-cellular localization

Systematic evaluations of hepoxilin hydrolase activity distributions have not been performed to date. However, this activity appears to be widely distributed in mammals, as indicated by the presence or formation of trioxilins reported in liver [20], platelets [126,210], brain (homogenates, hippocampus and pineal gland) [211–214], rat aorta [215], skin [21,207,216], and pancreas [217,218].

2.3.2. Substrates

As indicated above, the hepoxilin EH appears to have a high substrate specificity for the hepoxilins, as opposed to either leukotriene A_4 or *trans*-stilbene oxide [20]. The hepoxilins are structurally classified into to groups as described in Fig. 4, the γ-hydroxy epoxides separated by *trans* olefins (i.e. hepoxilin As), and the *alpha*-hydroxy epoxides (i.e. hepoxilin Bs), while the total olefin count in the molecule are indicated by numerical subscripts (i.e. arachidonic acid derived hepoxilins are A_3s and B_3s) [219]. These compounds are produced from lipid hydroperoxides either by autooxidative interaction with ferrous proteins [220] or enzymatically [221] by the action of hydroperoxide isomerases acting on lipid hydroperoxides [206] as shown in Fig. 5. Hepoxilins produced from the isomerization of 12-hydroperoxy eicosatrienoic acid (12-HPETE) and 15-HPETE have been reported [54,206,210,222]. In addition, the corresponding hepoxilins and trioxilins from docosahexenoic acid are produced in rat tissues [223].

2.3.3. Regulation

To the best of our knowledge, no information exists on the regulation of the hepoxilin epoxide hydrolase. Considering the implication of hepoxilins as regulators of numerous areas of physiology, this area is a deserving one for the focus of future research.

2.3.4. Physiological roles

The hydrolysis of hepoxilins appears to play a vital role in mammals by rapidly transforming these compounds to their corresponding trihydroxy metabolites, trioxilins. This action is, however in competition with both glutathione conjugation [212,215,224,225] in various tissues and

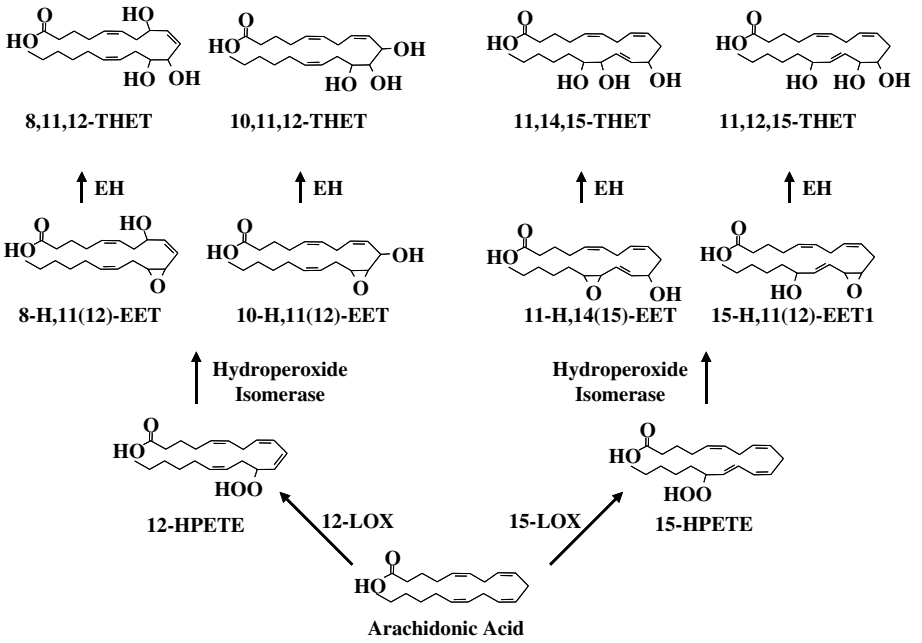


Fig. 5. Schematic representation of the enzymatic formation of hydroxy, epoxy and trihydroxy metabolites of arachidonic acid along with associated nomenclature: LOX: lipoxygenase; HPETE: hydroperoxyeicosatetraenoic acid; HEET: hydroxy eicosatrienoic acid, THET: trihydroxy eicosatrienoic acid.

omega-hydroxylation in neutrophils [226,227], and the relative roles of each pathway are unknown in specific tissues. However, unlike the trioxilins, the glutathione adducts retain their activity [212,225]. To date the trioxilins have been reported as degradation products of hepoxilins lacking the biological activity of the parent compounds [209,228]. However, it is interesting to speculate that the trioxilins themselves may have biological activities distinct from their precursors, as is true for other epoxide hydrolysis products [229,230]. Consistent with this hypothesis is the fact that both hepoxilins and trioxilins are actively incorporated into phospholipids [21]. Phospholipid hydrolysis using either alkaline conditions or phospholipase A2 produced similar hepoxilin and trioxilin quantities, indicating their preference for the sn-2 position of glycerophospholipids [21]. Regardless of whether the trioxilins themselves are bioactive, the activity of the hepoxilins and the identification of trioxilins in multiple tissues suggest that the hepoxilin EH plays a role in a number of physiological systems.

2.3.4.1. Platelet aggregation and inflammation. At the cellular level, hypotonic swelling of platelets induces hepoxilin A_3 formation, which is responsible for swelling reversal [231,232]. The addition of 1 μM 3,3,3-trichloropropene-1,2-oxide (TCPO), a confirmed hepoxilin epoxide hydrolase inhibitor, enhances the hepoxilin potency in this system [231]. While this inhibitor is quite toxic and produces transient inhibition of the mEH at these concentrations, inhibition of the sEH requires mM concentrations of TCPO [233]. Short duration shear stress has also been reported to result in hepoxilin formation, inhibiting platelet aggregation [234], by apparent interaction with thromboxane receptors [235–238].

Like the sEH, the hepoxilin hydrolase appears to have a role in the regulation of inflammatory events. Neutrophils can synthesize hepoxilins, where they bind tightly and selectively to the intracellular face of neutrophil membranes [239,240] causing an initial rapid rise in intracellular calcium followed by a slow decline to a plateau [241,242]. This bimodal effect on calcium was caused by an initial release of calcium from the endoplasmic reticulum, followed by a tight sequestration of the cation in the mitochondria [243], and is preceded by the receptor mediated activation of phospholipase C and A2 [244]. The hepoxilins also inhibit calcium mobilization in neutrophils stimulated by various inflammatory agents including formyl-methionyl-leucyl-phenylalanine, platelet-activating factor and leukotriene B_4 [245]. In addition, these compounds can elicit neutrophil shape change [246] and is a potent chemotactic agent [247] suggesting a role in neutrophil activation. Therefore, the identification of hepoxilins as endogenous products of neutrophils, their ability to modulate the function of these cells, their ability to enhance vascular permeability [248] and the elevated formation of hepoxilins and trioxilins by skin under inflammatory insult [207,249] suggest a role for the hepoxilin epoxide hydrolase in the modulation of inflammatory responses.

2.3.4.2. Smooth muscle tone. The hepoxilins have been reported to have direct actions on smooth muscle tone. Hepoxilin A_3 sensitized both thoracic aorta and portal vein from rats to the contractile effect of noradrenalin, more potently than the peptide-analog [250]. In addition, guinea pig trachea contraction induced by the potent bronchoconstrictor neurokinin A was potentiated by hepoxilins and unaffected by trioxilins [228], suggesting that the hepoxilin epoxide hydrolase activity is critical for resolving/balancing bronchiospastic conditions mediated through the hepoxilins.

2.3.4.3. Carbohydrate metabolism. On a systemic level, hepoxilins are involved in the regulation of insulin signaling, suggests that the hepoxilin epoxide hydrolase also plays a role in this critical homeostatic function. Early in the investigation of hepoxilin actions, these compounds were identified as insulin secretogagues [251]. Consistent with this role, hepoxilins were found as metabolic products of pancreatic islets of Langerhans [217,218]. While the similarity between the effects of leukotriene C_4 and hepoxilin A_3 on insulin secretion [252] suggests this function is mediated through peptidyl-hepoxilins, the injection of arachidonic acid produced a large increase in the blood concentrations of thromboxane B_2 and trioxilin A_3 within 1 min [253]. Furthermore, the mean concentration of this these products appeared greater in the diabetic rat than in the normal rat [253], suggesting an integral role for the hepoxilin epoxide hydrolase. Intra-arterial hepoxilin administration induces insulin secretion in the fed, but not fasted rat [254]. The hepoxilin pathway has also been proposed to have a neuromodulatory role in the central nervous system [255,256] and are potentiators of neurite regeneration [257].

2.3.4.4. Summary and future perspectives. Therefore, the hepoxilin epoxide hydrolase activity in vivo likely plays a modulatory role in inflammation, vascular physiology, systemic glucose metabolism, neurological function, and possibly tissue repair post injury. While the hepoxilin hydrolase appears to be a distinct enzyme, the substrate specificity of the sEH, and particularly the demonstrated ability of the plant sEHs to hydrolyze hepoxilins, suggests that this enzyme may also participate in this function. Therefore, the purification and cloning of the hepoxilin hydrolase will be critical to truly distinguish the physiological role of these two enzymes. It is also of some interest

that the recently reported cyclopropyl hepoxilin analogs reported as novel thromboxane receptor antagonists with a host of interesting properties [235,237,258,259] may also be competitive inhibitors of epoxide hydrolase activity [260].

2.4. Leukotriene A_4 hydrolase

Leukotriene A_4 hydrolase (LTA_4 hydrolase) is a bifunctional zinc metaloprotease [261], which displays both epoxide hydrolase and aminopeptidase activities [262]. Interestingly, these two catalytic sites share a common carboxyl recognition site and binding of 5(S)-*trans*-5,6-oxidoeicosatetra-(7*E*,9*E*,11*Z*,14*Z*)-enoic acid, i.e. leukotriene A4 (LTA_4), inhibits peptidase activity [263]. Lekotriene A_4 is synthesized from the 5 lipoxygenase product 5-HPETE. This relatively unstable epoxy lipid can either be converted to peptidyl leukotrienes by leukotriene C_4 synthase [264], hydrolyzed by sEH to a 5,6-dihydroxy metabolite [84,265], or converted to the 5(S),12(R)-dihydroxy eicosatetra-(6*Z*,8*E*,10*E*,14*Z*)-enoic acid metabolite leukotriene B_4 (LTB_4) by LTA_4 hydrolase [266].

LTA_4 hydrolases have been cloned from yeast (*Saccharomyces cerevisiae*) [267], frogs (*Xenopus laevis*) [268], and mammals: mouse (*Mus mus*) [269,270], rat (*Ratus norwiegicus*) [271], human (*homo sapien*) [272,273]. Recently, a crystal structure of the human LTA_4 hydrolase was obtained and new insights into the catalytic mechanism of the enzyme have been elucidated [274–276].

2.4.1. Tissue distribution and sub-cellular localization

The LTA_4 hydrolase is a cytolsolic enzyme found both in heamopoietic [277,278] and paranchimal tissues [279]. The presence of LTA_4 hydrolase activity has been documented in various organs and cell types using combinations of activity and histochemical detection. In the blood stream LTA_4 hydrolase occurs in neutrophils [278], macrophages [280], erythrocytes [279,281], and platelets [282], but not eosinophils, which release the peptidyl leukotriene LTC_4 directly [283]. This enzyme is also found in the liver [279], lung [284], kidney [285], heart [270], adrenal cortex [270], gastro intestinal tract [286], spleen [270], skin [287,288], reproductive organs [289], cartilage [290], and brain [291]. Within these various organs, the enzyme has been localized to tissue-resident leukocytes [270,287,292], pulmonary [270,293], gastrointestinal [286], and corneal epithelium [294], skin epidermal and Langerhan cells [288], renal mesangial cells, all nephron segments, and collecting tubules [270,295,296], vascular endothelium [279,281], vascular smooth muscle [281], seminal vesicles [270], large luteal ovarian cells [289], and hepatocytes [270]. In addition, the LTA_4 hydrolase may also be found extracellularly, as demonstrated by its presence in cell free bronchiolar alveolar lavage fluids [297], however this may simply reflect alveolar neutrophil infiltration and lysis. Two unique LTA_4 hydrolase mRNA splice variants have been reported that are constitutively expressed in multiple tissues [281], however it is not known if each of these variants are translated into a functional protein. It is of interest however that a related protein, aminopeptidase B, may also show weak LTA_4 hydrolase activity [298,299], and that the LTA_4 hydrolase isolated from pulmonary epithelium and neutrophils show a differential sensitivity to pharmacological agents [300].

2.4.2. Substrates

As the name suggests, LTA_4 hydrolase displays a high degree of substrate specificity for LTA_4. The enzyme requires the presence of a free acid function and prefers a 7,9-*trans*-11,14-*cis* tetraene

configuration in its substrates [301]. While the enzyme will transform the corresponding LTA_3, containing a 7,9-*trans*-11-*cis* triene structure, to LTB_3, it does so at ~30-fold lower rate [302] or not at all [303]. LTA_3 has also been described as a potent LTA_4 hydrolase suicide substrate [304]. Similarly, LTA_5 is a hydrolyzed at a 4-fold lower rate and acts as an inhibitor of LTA_4 hydrolysis [305]. Substrate mediated inactivation studies using functional mutants resistant to inactivation suggest that substrate inactivation of LTA_4 hydrolase is reliant on the substrate affinity for the catalytic site [306].

While a definitive description of the endogenous peptide substrate for the LTA_4 hydrolase has yet to be demonstrated, this protein metabolizes arginyl peptides with high efficiency and catalytic transformations are greatest with tripeptides [262]. In addition, opioid peptides including met5-enkephalin, leu5-enkephalin, dynorphin1-6, dynorphin1-7, and dynorphin1-8 have been described as endogenous competitive inhibitors and substrates of the aminopeptidase site [307]. The cleavage of N-terminal tyrosines from the enkephalins inactivated these analgesic peptides [307].

2.4.3. Regulation

The regulation of LTA_4 hydrolase is achieved at transcriptional, post-translational, and functional levels. In human polymorphonuclear leukocytes (PMNs), interleukin-4 and interleukin-13 enhanced A23187-stimulated increased mRNA expression and protein synthesis of LTA_4 hydrolase, but not those of cPLA(2) or 5-LO [308]. In keratinocytes, LTA_4 hydrolase protein expression is down regulated by the anti-inflammatory agent cyclosporine A, but not 1,25-dihydroxyvitamin D_3, all-*trans* retinoic acid, eicosatrienoic acid, dexamethasone, interferon-γ or methotrexate [309,310]. In addition, LTA_4 hydrolase expression is stimulated by human chorionic gonadotropin in leutial cells of the ovary during early pregnancy [289]. It is also of interest that in both fibroblasts and esophageal epithelium, carcinogenic transformations lead to induction of LTA_4 hydrolase gene expression [311,312]. Therefore the regulation of LTA_4 hydrolase expression suggests the presence of specific transcriptional regulatory binding sites in the 5′-flanking region of this gene.

Cloning of the LTA_4 hydrolase 5′-flanking region revealed the presence of several transcription-factor consensus sequences, including a phorbol-ester-response element (AP2) and two xenobiotic-response elements [273,313]. These findings are consistent with earlier studies investigating the effects of phorbol esters on LTB_4 production indicating that LTA_4 hydrolase is activated by protein kinase C-dependent phosphorylation [314]. In fact, it has since been demonstrated that basal LTA_4 hydrolase in vascular endothelium exists in an inactive, phosphorylated state [315]. Phosphorylation at Ser415 is accomplished by protein phosphatase 1 in the presence, but not absence, of an LTA_4 hydrolase peptide substrate [315], suggesting dynamic regulation of LTB_4 production by an intracellular kinase/phosphatase interaction. These findings suggest that the depressed LTA_4 hydrolase activity occurring in conjunction with stable protein levels in psoriatic skin lesions [316] may be a result of post-translational phosphorylaton of the LTA_4 hydrolase.

The LTA_4 hydrolase is inhibited by its substrates, a process which limits production of LTB_4 in LTA_4 synthase containing cells [277]. In the circulatory system and many tissues, this process is over come by leukocyte-resident cell interactions, where transcellular delivery of LTA_4 from leukocytes allows the accelerated production of LTB_4 [277]. It has also been noted that under conditions of essential fatty acid deficiencies, the production of a lipoxygenase metabolites result in the inhibition of LTA_4 hydrolase, decreasing basal LTB_4 production below what would be

expected from arachidonic acid depletion [317,318]. Whether this is due to the presence of an inhibitory substrate, or in fact an alteration in the phosphorylation state of the enzyme has not been clearly investigated.

Finally, the peptidase activity of LTA_4 hydrolase is stimulated by chloride ions, and kinetic analysis of the results suggested the presence of an anion binding site [319]. This peptidase activity is in turn retarded by preincubation of the enzyme with LTA_4, which could prolong the activity of endogenous opioids during inflammatory episodes [320].

2.4.4. Physiological role: inflammatory regulator

The current understanding of LTA_4 hydrolase clearly indicates a pro-inflammatory role for this enzyme [321–323]. The synthesis of LTB_4 has been linked to the pathophysiology of various inflammatory diseases of the skin [266,324], joints [325], bowels [325], lung [326], and kidney [327–329]. LTB_4 is a potent chemokine which stimulates leukocyte degranulation [330], has leukotactic properties [331], and stimulates DNA synthesis, cell replication and IgG secretion [332]. Furthermore, LTA_4 hydrolase-deficient mice are resistant to platelet-activating factor, suggesting that LTB_4 is a mediator of systemic shock [322]. Mechanistically, it has been shown that LTB_4 can regulate leukocyte activation by modulating polyisoprenyl phosphate signaling. Specifically, LTB_4 receptor stimulation activates phospholipase D and concurrently reduces presqualene diphosphate production, reducing this compounds blockade of leukocyte activation and superoxide anion generation [333].

LTA_4 hydrolase also plays a role in female reproduction. The sensitivity of LTA_4 hydrolase to human chorionic gonadotropin, and the enhanced expression of this enzyme during corpus leuteum formation suggest the involvement of LTB_4 in luteal cells during early pregnancy [289].

A functional role for the peptidase activity of LTA_4 hydrolase is still elusive. However, the ability of this enzyme to inactivate enkephalins by cleavage of the terminal tyrosine residues is intriguing [320]. The finding that inactivation of the LTA_4 hydrolase by phosphorylation is accomplished only in the presence of a peptidase substrate [315] supports a role for the enkephalins in the resolution of inflammation by preventing LTB_4 production. The peptidase activity is in turn retarded by preincubation of the enzyme with LTA_4, prolonging the activity of endogenous opioids during inflammatory episodes [320]. The inactivation of these analgesic peptides during inflammatory stimulation provides a consistent role for both catalytic activities in the regulation of inflammatory events.

3. Membrane associated epoxide hydrolases

3.1. Microsomal epoxide hydrolase

Historically, the microsomal epoxide hydrolase was the first EH characterized and isolated from mammalian liver [334–336]. The cDNA of the mEH has been isolated from several species including rat and human [337,338] and the corresponding enzymes have been expressed in different transgenic systems [339–342]. The mEH protein is made of 455-amino acid residues corresponding to a ~50 kDa protein [343], with a strongly hydrophobic transmembrane anchor of approximately 20 residues at the N-terminal [344,345]. The C-terminal domain, which contains

the catalytic residues, is homologous to a haloalkane dehalogenase, like the sEH [18,34]. Recently, a sEH from the fungus *Aspergillus niger* was found homologous to the mammalian mEH, but without the N-terminal anchor [346]. This fungal enzyme was recently crystallized [347]. In humans, the mEH is the product of the *EPXH1* gene on chromosome 1 [348]. Several single nucleotide polymorphism sequences were identified in human [349] and have been found in association with the onset of several diseases and cancers [350–353].

3.1.1. Tissue distribution and sub-cellular localization

Like the sEH, the mEH has been found in nearly all mammalian tissues that have been evaluated [10]. Early investigations by Oesch and collaborators reported the detection of mEH in 26 different rat organs and tissues [354]. While mEH from animal livers has been primarily studied, mEH was also isolated from human adrenal glands [355], sinovial tissues [356], follicles isolated from mouse ovaries [357], and in pulmonary bronchial epithelium [358]. Considering the whole animal, mEH activity is generally the highest in liver, with lower yet similar levels in testis, lung and heart [110]. However, the relative levels vary with environmental exposures, sex and age (see [10] and [359] for reviews). For instance, a 63-fold interindividual variation in mEH levels has been reported in human livers [360].

It should be noted that in certain organs the mEH is localized within specific cell types, such that whole organ measurements do not necessarily reveal a localized high concentration of the enzyme. For example, while ubiquitously distributed in cerebral tissues, mEH is primarily localized in glial as opposed to neuronal cells [361], and has elevated activities in tissues which function as blood- and cerebrospinal fluid-brain barriers [362]. In particular, the mEH activity in the choroid plexus approach or exceed those of the liver. It has been hypothesized that the choroid plexus my serve both hormone generation and detoxification functions for the brain, in a fashion similar to that of the liver for the rest of the body [362]. Furthermore, mEH activity [363] and gene expression [364] has been detected in human blood cells, especially in lymphocytes and monocytes, underlying the necessity to exsanguinate tissues before any mEH measurements. Finally, the expression of mEH has been reported in numerous cancerous and primary cell lines (see [10] for review).

As the name implies, the mEH has been primarily isolated and characterized from microsomal preparations [365]. As a precautionary note, EH activities in microsomes fractions should not be confused with mEH, because prepared microsome fractions also contain the ChEHs [366] and sEH activity [106,109]. In addition, mEH activity has been found in the cytosol of neoplastic human livers [367]. In liver, mEH is found on the smooth endoplasmic reticulum [368], but has also been reported in association with the plasma membrane [369,370]. Interestingly, the topological orientation of mEH appears to be different in the ER, where the catalytic C-terminal domain faces the cytosol [371], and in the plasma membrane where the C-terminal faces the extra-cellular medium [372]. Sequence analyses suggest that the association of the mEH with the membranes is due to the presence of an N-terminal transmembrane anchor [344,345]. However, the removal of this anchor does not result in a soluble protein [345], suggesting a strong hydrophobic interaction of this enzyme with the membrane. Furthermore, mEH was reported to be tightly associated with phospholipids [373,374], to be a subunit of a Na^+-dependent bile acid transport system [369] and to represent a high affinity tamaoxifen binding site [375,376].

3.1.2. Substrates

The mEH is well recognized as a key enzyme in the metabolism of environmental contaminants [377]. Consistent with this fact, the majority of studies investigating the mEH substrate specificity have focused on its role in xenobiotic transformations. Early reviews provide a good summary of these results [365,378,379]. Most of these studies used microsomal preparations, rather than purified enzymes, and available competitive substrates, putting some shade on the interpretation of the results since other hydrolases co-exist in such preparations [380]. Regardless, these studies suggest that the mEH prefers mono- and *cis*-1,2-disubstituted epoxides, while *gem*-di-, *trans*-di-, tri- and tetra substituted epoxides are either low turnover substrates or inhibitors [365]. As shown in Fig. 6, the mEH can metabolize a broad array of epoxide containing compounds. These include aliphatic epoxides (e.g. butadiene oxide, 1,2-epoxyoctane), and polyaromatic oxides (e.g. phenanthrene oxide, carbamazepine oxide, benzo(*a*)pyrene-4,5-oxide) [11,17]. Styrene and *cis*-stilbene oxides are still widely used as mEH surrogate substrates [365].

More central to this review, the mEH dependent metabolism of endogenous lipids has also been reported. In particular, androstene oxide (16α,17α-epoxyandrosten-3-one) and estroxide (epoxyestratrienol) were reported as endogenous substrates of mEH [381]. While epoxy-fatty acids such as epoxy-stearic acid, are relatively poor substrates for mEH compared to sEH [119], the former enzyme hydrolyzed this compound with a high enantioselectivity, while the latter did not [33]. In addition, epoxide-containing glycerol-phospholipids are poor substrates for the mEH [382].

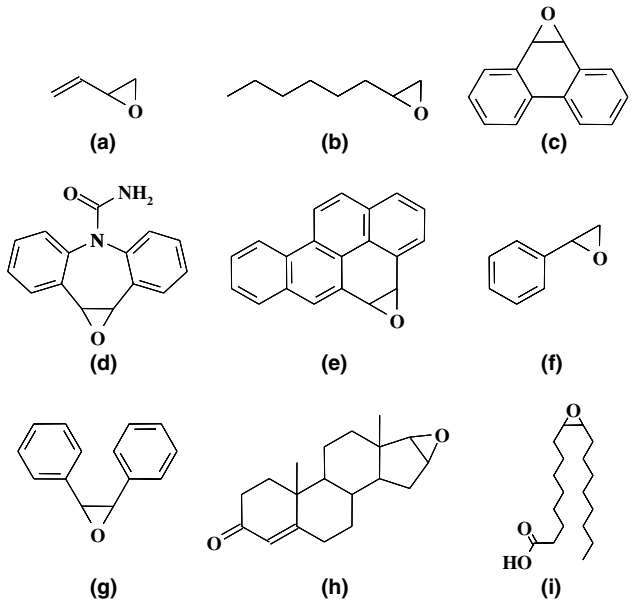


Fig. 6. The structures of reported mEH substrates. (a) butadiene oxide; (b) 1,2-epoxyoctane; (c) phenanthrene oxide; (d) carbamazepine oxide; (e) benzo(*a*)pyrene 4,5-oxide; (f) styrene oxide; (g) *cis*-stilbene oxide; (h) androstene-16,(17)-oxide; (i) 9(10)-epoxy octadecanoic acid.

3.1.3. Regulation

The regulation of mEH can occur at the transcriptional, translational [11] or post-translational level [383]. The induction of mEH has been well studied in animals, but the confidence with which to extrapolate these results to humans is not known. In rodents, mEH can be induced by a variety of compounds that increase the rate of gene transcription [384]. The list of known inducers include phenobarbital, methylcholanthrene, polychlorinated biphenyls, *trans*-stilbene oxide [385,386], peroxisome proliferators [387], radiation [388], heavy metals [11], and certain steroids, including estroxide and its precursor estratetraenol [389]. Complicating matters, the effect of each inducer is variable upon age, sex, strain, and species [387,390]. For instance in the rat, the induction of mEH by methadone is dependent on sexual hormones, in that castration results in methadone-dependent suppression of mEH, while ovarectomy yields methadone-dependent induction of this activity [391]. More recently, the association of mEH with various cytochrome P450s was shown to affect the rate of substrate hydrolysis in vitro [383]. Of the tested P450s, CYP2C11 appeared to play the greatest role in the association/activation of mEH.

Suppression of mEH activity has also been reported. Inducing a diabetic state using either alloxan or streptozotocin lead to a 71% reduction in hepatic mEH activity in rats [142]. This depressed mEH activity was restored by insulin administration [142]. Recently, mEH activity was also reported to increase in rat hepatocyte cultures following insulin exposure [392]. A forced fast of 2–5 days also reduced mEH activity by ~60% [142]. The expression of the mEH gene was also severely downregulated in intra-epithelial lymphocytes from mice receiving total parenteral nutrition [393]. Suppression of mEH gene expression has also been reported using dexamethasone, gadolinium chloride, acriflavine and lipopolysaccharide [394–398], as well as experimental traumatic injury [399]. Further glucocorticoid-dependent repression has been directly attributed to interactions with the 5′-flanking sequence of the *EPXH1* gene [398]. Adrenylectomy in rats resulted in elevated mEH levels, which were reversed by dexamethasone, but not deoxycorticosterone, supporting a role for the hypothalamus–pituitary–adrenal axis in mEH regulation [400]. In addition, hypophsectomy (i.e. pituitary gland removal) induced hepatic mEH activity in both males and females, and growth hormone supplementation reduced this activity below that of sham-operated animals [152]. These results suggested that the mEH in the liver is under suppressive control by the pituitary and that growth hormone may be the causal hormone involved in the sexually dimorphic expression of this enzyme [152].

Preliminary tests on human primary hepatocyte cultures indicate that the human mEH gene may only be modestly responsive to chemical exposures [401]. Furthermore, in humans the presence of two single nucleotide polymorphisms in exons 3 and 4 [349,402] and several others in the 5′-flanking region [403] seems to effect the regulation of mEH gene transcription [11]. While the genetic variants have a lower specific activity than the wild type, activity levels in human livers were found to be independent of the polymorphism, indicating that the genetic variations only modestly impact the resulting mEH specific activity in vivo [23].

Developmentally, mEH gene expression has been reported to increase steadily in the liver of man and correlate strongly with gestational age and protein expression and activity [404]. Expression levels do not appear to correlate with either gestational age, activity, or immunoreactive protein in other inspected tissues [404,405].

3.1.4. Physiological roles

While well recognized as a critical enzyme in xenobiotic detoxification, the implication of the mEH-dependent metabolism of endogenous lipid substrates is less well defined. Despite the fact that mEH null mice do not present an obvious phenotype without exposure to pro-carcinogens [406], there are several points indicating an endogenous role for this enzyme, beyond xenobiotic metabolism.

3.1.4.1. Cytoprotection. Epoxides are strained three-membered cyclic ethers, and when combined with electron withdrawing structures, can become highly reactive electrophilic mutagens, carcinogens or cytotoxins [365]. The conversion of epoxides to diols by the mEH generally results in less mutagenic or carcinogenic compounds [334]. This detoxification role of mEH likely predominates in the liver [10], and perhaps the choroids plexus of the brain [362], but mEH is also involved in the extra-hepatic metabolism of these agents, such as pulmonary naphthalene metabolism [407]. The protective role of mEH from xenobiotics was illustrated in the case of a man with a defect in mEH expression suffering from acute and severe phenytoin toxicity [408]. In addition, sorbinal hypersensitivity may also be related to a reduced mEH activity [409].

Interestingly, in the case of some polyaromatic compounds, such as benzo(a)pyrene 4,5-oxide, dihydrodiol formation can stabilize bay-region epoxides, increasing the mutagenic and carcinogenic potential of the product [410,411]. This pro-carcinogenic role of mEH was illustrated in mEH-null mice [406]. Furthermore, in human populations, mEH polymorphisms have been associated with the onset of numerous cancers [351–353,412–414] and the mEH, but not ChEH is upregulated in hyperplastic tissues [380]. In some populations, the role of mEH in xenobiotic metabolism may also be linked to the relationship between mEH polymorphism and emphysema [350,415,416] or Crohn's disease [417].

3.1.4.2. Steroid metabolism. Numerous lines of evidence suggest that the mEH may play a role in steroid biosynthesis or metabolism. Epoxy-steroids are known endogenous compounds [418], the mEH is found in steroidogenic tissues [110,357,381,419,420], mEH inhibitors interfere with testosterone to estradiol conversion [421], potential relationships have been found between mEH polymorphism and spontaneous abortion [422], preeclampsia [423] and polycystic ovary syndrome [424], the mEH has been identified as a subunit of an anti-estrogen binding site [375,376], and the tested epoxy steroids are in fact hydrolyzed by mEH to their corresponding *vicinal*-diols [381,389]. In particular, the epoxides of estratetraenol and androstadienone, estroxide and adrostene oxide, are good mEH substrates [381]. The endogenous roles of epoxy steroids are not well known, however these compounds may be toxic. For instance, estrogen epoxide has been hypothesized as a critical breast cancer initiation factor [425], whose formation is inhibited by tamoxifen treatment [426]. Therefore, mEH may be important in the cellular protection from steroid metabolites, as it is in the metabolism of epoxidized xenobiotics [9].

3.1.4.3. Other. Beyond these direct roles in steroid metabolism and toxicant transformations, the mEH may also have roles in bile acid transport and cellular responses to glucose metabolism. The mEH has been described as mediating the Na^+-dependent transport of bile acid into hepatocytes [369,427]. This role of mEH appears dependent on its expression on the surface of cells [428], and the enzyme is apparently part of a multi-protein transport system [429]. However, the mechanism

by which mEH participates in bile absorption is not yet known. Interestingly, mEH expression was found greatly reduced in a patient with hypercholastanemia, suggesting that the absence of mEH may impair the hepatic re-absorption of bile acids, leading to their accumulation in the blood and the onset of this disease [430]. Hormones which regulate blood glucose, including insulin and glucagon, also affect the expression of mEH in hepatocytes cultures [392], and imbalance in these hormones are well known factor in the occurrence of polycystic ovary syndrome [431,432].

3.2. Cholesterol epoxide hydrolase

The cholesterol epoxide hydrolase (ChEH) is the other known EH located in the microsomal fraction in mammals [366,433,434]. This enzyme has yet to be purified to homogeneity, and neither the corresponding cDNA nor the gene has been cloned. Consequently little is known about the biochemistry and molecular biology of the ChEH [11]. While the exact mechanism of ChEH is not well known, several lines of evidence suggest that the catalytic mechanism differs from those of the sEH and mEH. First, the enzyme was found to be too small to be an α/ß-hydrolase fold enzyme [17,434]. Furthermore, unlike mEH or sEH, ChEH appears to hydrolyze cholesterol oxides via a positively charged transition state [435] without the formation of a covalent intermediate [19]. These findings suggest a one step acidic mechanism similar to the one described recently for the limonene-1,2-epoxide hydrolase from *Rhodococcus erythropolis* [436].

3.2.1. Tissue distribution and sub-cellular localization

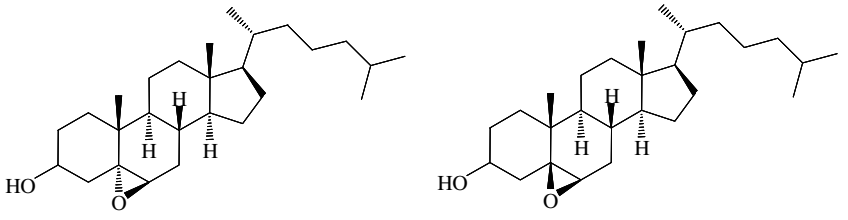
Like the mEH, ChEH is widely distributed in mammals, with all tissues tested showing activity. The ChEH specific activity of liver microsomes is reported as ~5-fold higher than that of the kidney, lung, testis, spleen and other organs examined [437].

3.2.2. Substrates

The ChEH is highly specific for cholesterol-5,6-oxides (Fig. 7) [366]. The enzyme shows a 5-fold preference for the *alpha*- versus the *beta*-diastereomer [438].

3.2.3. Regulation

Induction of the ChEH has been reported in rodents exposed to the anti-hyperlipidic compound clofibrate [439], a known PPARα agonist. Unlike the mEH, ChEH was not elevated in



alpha-5(6)-epoxy-cholestan-3-ol *beta*-5(6)-epoxy-cholestan-3-ol

Fig. 7. Structure of cholesterol-5,6-oxides.

hyperplastic tissues [380]. Furthermore, ChEH is inhibited by its primary product, cholestanetriol [438], as well as ketocholestanols, and several cholesterol derivatives [435,440].

3.2.4. Physiological roles

While a definitive physiologic role of ChEH is not known, much is known about the biological activity of the substrate and product of this enzyme. Cholesterol oxides and triols are naturally occurring components of human plasma, where the primarily occur as unesterified lipids [441]. The epoxides appear to be formed through interactions with lipid hydroperoxides [442] as opposed to a monooxygenase mediated process.

3.2.4.1. Cytoprotection vs vascular homeostasis. The weak mutagenicity of cholesterol oxides [443] suggests that the ChEH could play a role in protecting cells from these steroid toxicants. However, the exceptional chemical stability of the cholesterol epoxide suggests that this mutagenic effect is not through nucleophilic adduct formation. In addition, the corresponding cholestantriols are themselves cytotoxic [444], and are associated with increased lipid peroxidation [445] and disruption of actin microfilaments [446], therefore, the epoxide hydrolysis may actually represent an activation event. Both cholesterol epoxide and triol have also been shown to alter various aspects of vascular function [447]. These agents inhibit the production of the vasodilator prostacyclin [448,449], reduce platelet adhesion to endothelial mono-layers [449], and down regulate expression of the LDLR gene [450]. Together, these reports suggest that the ChEH may play a critical role in the regulation of vascular homeostasis.

3.2.4.2. Phospholipid biosynthesis. The cholestantriol has also been reported to activate cytidyl transferase, increasing phospholipids synthesis and altering phospholipid head group composition [451]. These changes in phospholipids may ultimately affect membrane properties and activity of membrane bound enzymes [451]. Therefore, the ChEH may be integrally involved in the regulation of phospholipid biosynthesis, and such a role is consistent with its induction by PPARα agonists.

3.3. Juvenile hormone EH – the characterized insect EH

The juvenile hormone epoxide hydrolase (JHEH) is an enzyme involved in the metabolic degradation of juvenile hormones (JHs), a series of structurally similar terpenoid esters containing terminal tri-substituted epoxides derived from farnesyl (Fig. 8). Regulation of this hormone governs multiple aspects of insect growth and development. The degradation of these epoxy terpinoids in insects has been well studied, and two hydrolytic pathways are known. The methyl ester is cleaved by a soluble esterase, JH esterase, and the tri-substituted epoxide is hydrolyzed by a microsomal enzyme, JHEH [452,453]. Each of these metabolic steps alters, if not eliminates JH activity [452,453]. The relative role of epoxide hydration and ester hydrolysis in JH catabolism vary with species and insect life stage [454]. For instance, in *Drosophile virilis*, the esterase pathway appears to dominate the regulation of the JH titer, however JHEH appears to assume this role in *Drosophila melanogaster* [455]. While the biological consequence of ester hydrolysis can be reversed by methyltransferases, epoxide hydrolysis and further conjugation as phosphates [456,457] represent irreversible degradation.

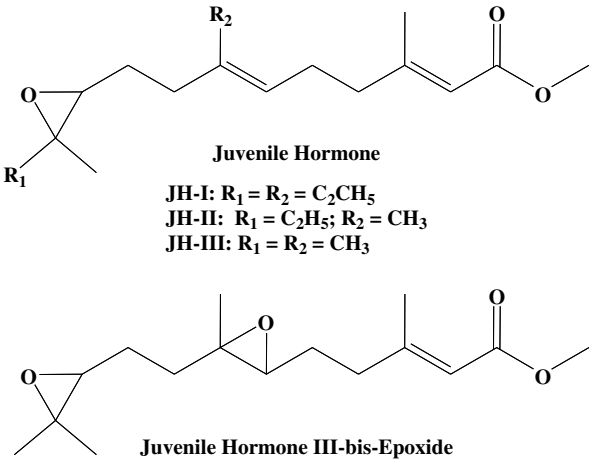


Fig. 8. Structure of insect juvenoids.

Studies of the metabolism of JHIII and JHIII-bis-epoxide suggested the existence of a JHEH [127]. The JHEH has since been cloned and expressed from the tobacco hornworm (*Manduca sexta*) [15,458–460], the cabbage looper (*Trichoplusia ni*) [461], and the cat flea (*Ctenocephalides felis)* [462]. Each of these JHEHs are roughly 50-kDa and show high homology to the rat microsomal EH [462]. Evidence for multiple EHs in the insect genome have been reported in both the cat flea [462] and cabbage looper [16].

3.3.1. Tissue distribution and sub-cellular localization

The JHEH is a microsomal enzyme which has been observed in developing oocytes, fat body, and midgut epithelium of the adult cat flea in immunohistochemistry experiments using affinity-purified rabbit polyclonal antibodies [462]. JHEH has also been purified from the eggs [458,460] and Malpighian tubules [459] of *M. sexta*.

3.3.2. Substrates

As shown in Fig. 8, four JHs have been identified to date, including JHI, II, and III along with their corresponding 6(7)- or bis-epoxides [463]. The different structural variants are separated between insect genera, with JHIII being the most common. The substrate specificity of the JHEH has not been thoroughly investigated; however a preliminary study with the recombinant *M. sexta* JHEH suggested that this microsomal enzyme is specific for the juvenile hormones [15].

3.3.3. Regulation

To date, little is known about the regulation of JHEH. In the cat flea, the expression of JHEH mRNA was relatively constant throughout the different larval stages, but was slightly elevated in the unfed adult flea [462]. However, JHEH activity was highest in the late larval, pupal, and adult stages [462], suggesting either altered rates of translation or post-translational regulation of enzyme activity.

3.3.4. Physiological roles

Although initially identified as a "factor" that keeps larval insects in the juvenile state, JHs and/or their metabolites have subsequently been shown to play critical roles in numerous insect life processes including development, metamorphosis, reproduction, diapause, migration, and metabolism [452,464]. For instance, in metamorphosis the reduction in its titer initiates development [464], while the same decline appears to stimulate oviposition of fertilized eggs in the adult [455]. These diverse roles in the insect life cycle suggest that the biosynthesis, transport, and degradation of JH and/or its metabolites are carefully regulated. It has become evident that the JHEH is in fact an important enzyme in the regulation of this insect hormone, and thus influences significant portions of insect physiology. Potent selective inhibitors active in vivo have dramatically advanced the study of the physiological roles for the sEH in mammals [106] and the JH esterase in insects [453]. While attempts have been made to produce such compounds for the JHEH [465,466], potent and stable inhibitors are still needed.

4. Conclusion

The production of epoxide containing metabolites in biological organisms has led to the evolution of a diverse array of EHs. It is particularly interesting that many, if not all of these epoxidized metabolites are bioactive and serve as signaling molecules in their host organisms. Thus, the EHs appear to have evolved as critical regulators within complex signal transduction pathways. While our understanding of these enzymes has expanded greatly since their discovery, many important questions remain to be answered, and doing so will allow a more refined interpretation of the importance and utility of various members of this enzyme class.

The bifunctional sEH and LTA_4 hydrolase pose unique challenges. While epoxide hydrolysis has been well studied in these enzymes, the true roles of the sEH phosphatase and LTA_4 hydrolase peptidase activities remains to be fully elucidated. Considering that each of these enzymes appears to constitute a useful therapeutic target for the treatment of inflammatory diseases, additional efforts are warranted to expand our understanding of these non-epoxide hydrolase functions. Continued investigations of sEH dependent metabolism in plant host defense are also warranted. In particular, direct evaluation of sEH as an endogenous regulator of jasmonate signaling may allow a more detailed understanding of the control of stress responses in these organisms. Similarly, efforts to elucidate the true importance of the mEH in steroid and glucose metabolism will undoubtedly expand our understanding of this enzyme beyond its function in xenobiotic detoxification. Furthermore, the JHEH is ripe for investigations designed to fully elucidate the metabolic and catabolic mechanisms of juvenile hormone control in insects. At present, the importance of the cholesterol epoxide hydrolase and hepoxilin hydrolase are suggested by the activity of there substrates, however our understanding is limited. Efforts to clone and express these enzymes will greatly improve our ability to ask decisive questions about their physiological roles. Therefore, in the coming years, additional attention focused on the EHs and their involvement in lipid metabolism will undoubtedly improve our understanding of an array of critical points of control in the physiological regulation of both plants and animals.

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