Toxicol Res. (2021) 37:285–292
https://doi.org/10.1007/s43188-021-00088-z

eISSN 2234-2753
pISSN 1976-8257

INVITED REVIEW

Regulation of cardiovascular biology by microsomal epoxide hydrolase

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Received: 23 December 2020 / Accepted: 6 January 2021 / Published online: 21 January 2021

Abstract

Microsomal epoxide hydrolase/epoxide hydrolase 1 (mEH/EPHX1) works in conjunction with cytochromes P450 to metabolize a variety of compounds, including xenobiotics, pharmaceuticals and endogenous lipids. mEH has been most widely studied for its role in metabolism of xenobiotic and pharmaceutical compounds where it converts hydrophobic and reactive epoxides to hydrophilic diols that are more readily excreted. Inhibition or genetic disruption of mEH can be deleterious in the face of many industrial, environmental or pharmaceutical exposures and *EPHX1* polymorphisms are associated with the development of exposure-related cancers. The role of mEH in endogenous epoxy-fatty acid (EpFA) metabolism has been less well studied. In vitro, mEH metabolizes most EpFAs at a far slower rate than soluble epoxide hydrolase (sEH) and has thus been generally considered to exert a minor role in EpFA metabolism in vivo. Indeed, sEH inhibitors or sEH-deficiency increase EpFA levels and are protective in animal models of cardiovascular disease. Recently, however, mEH was found to have a previously unrecognized and substantial role in EpFA metabolism in vivo. While few studies have examined the role of mEH in cardiovascular homeostasis, there is now substantial evidence that mEH can regulate cardiovascular function through regulation of EpFA metabolism. The discovery of a prominent role for mEH in epoxyeicosatrienoic acid (EET) metabolism, in particular, suggests that additional studies on the role of mEH in cardiovascular biology are warranted.

Keywords mEH · sEH · Cardiovascular · Epoxyeicosatrienoic acid · EPHX1 · Cancer

Introduction

Epoxide hydrolases catalyze the hydrolysis of electrophilic and potentially genotoxic epoxides to more soluble and less reactive diols. Mammalian microsomal epoxide hydrolase/epoxide hydrolase 1 (mEH/EPHX1) has broad tissue distribution and is capable of inactivating a wide variety of structurally dissimilar, highly reactive epoxides [1]. Thus, mEH plays an important role in detoxifying a large number of xenobiotic compounds [2]. It's role in detoxifying carcinogenic compounds suggests a role for mEH activity and/or *EPHX1* single nucleotide polymorphisms (SNPs) in the development of cancer [3].

mEH also metabolizes a variety of endogenous fatty acid epoxides. Cytochromes P450 can metabolize fatty acids such as arachidonic acid (AA), linoleic acid (LA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to biologically active epoxy-fatty acids (EpFAs) called epoxyeicosatrienoic acids (EETs), epoxyoctadecamonoenoic acids (EpOMEs), epoxyeicosatetraenoic acids (EpETEs) and epoxydocosapentaenoic acids (EpDPEs), respectively (Fig. 1) [4]. While the physiological effects of EETs have been most widely studied, EETs, EpETEs, and EpDPEs possess a variety of overlapping properties, including vasodilatory, anti-inflammatory, angiogenic and anti-apoptotic effects that are typically beneficial to cardiovascular homeostasis [4]. These epoxides have short half-lives and are inactivated to generally less biologically active vicinal diols by epoxide hydrolases [5]. In contrast, LA-derived EpOMEs have been termed leukotoxins as they have pro-inflammatory, cardiodepressive and/or cytotoxic properties, which may depend on epoxide hydrolase-mediated formation of their corresponding diols [6]. Soluble epoxide hydrolase/epoxide hydrolase 2 (sEH/EPHX2) plays a substantial role in EpFA hydrolysis and sEH inhibitors have been developed for the treatment of a variety of diseases, including diabetes,

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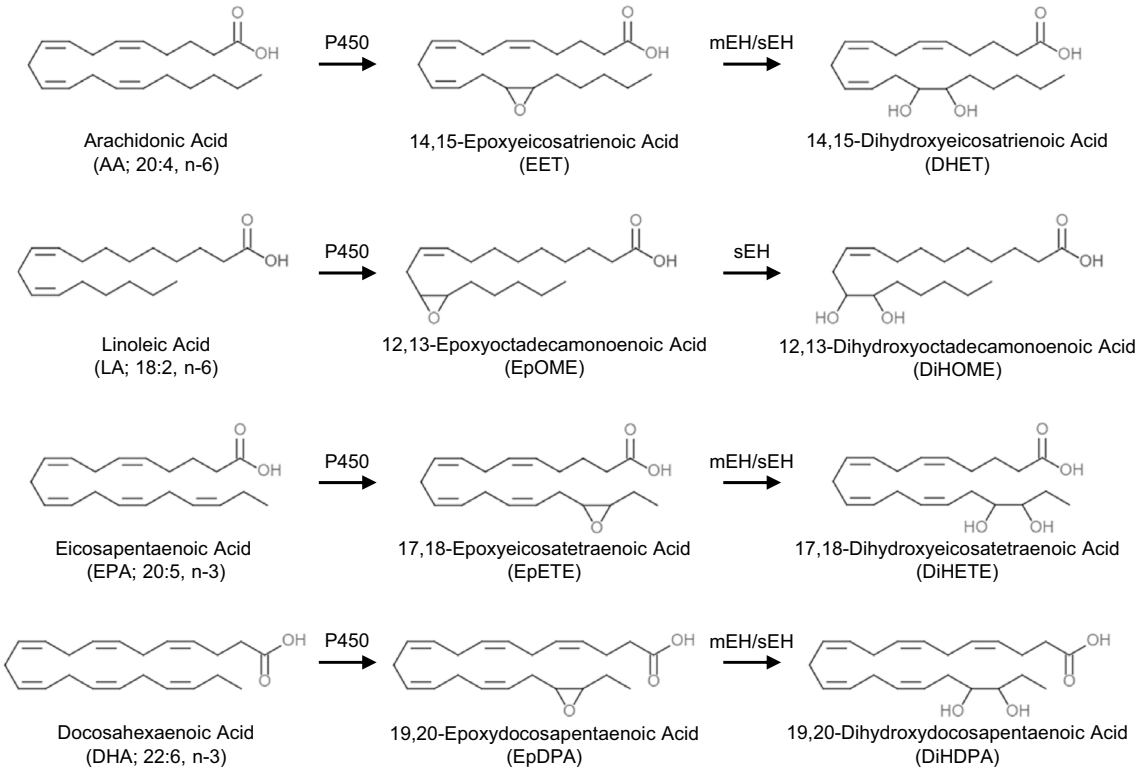

Fig. 1 Metabolism of fatty acids to bioactive epoxides and diols by cytochromes P450, mEH and sEH. Both omega-6 (n-6) and omega-3 (n-3) fatty acids are metabolized by cytochromes P450 to bioactive epoxides. Both mEH and sEH contribute to metabolism of AA-, EPA- and DHA-derived epoxides to generally less biologically active diols in vivo. In contrast, sEH is the dominant hydrolase for LA-derived epoxides in vivo

chronic obstructive pulmonary disease, and pain [5], and are currently in early phase clinical trials for some of these conditions.

A significant role of mEH in EpFA hydrolysis and cardiovascular physiology has only become evident in recent years [7, 8]. Both sEH and mEH were known to hydrolyze EpFAs in vitro; however, sEH metabolizes EETs at rates that are 10- to 100-fold faster than mEH [9, 10]. The preferred substrates for sEH are fatty acid epoxides with the epoxy moiety on the most omega-situated olefin (e.g., 14,15-EET and 12,13-EpOME), while mEH displays a preference for epoxides on mid-chain olefins (8,9- and 11,12-EET and 9,10-EpOME). Tissues from sEH-deficient mice have in 75–99% reduction in EET hydrolysis in vitro, and lesser, but significant decreases in plasma DHET levels [7, 10, 11]. Given the disparities in in vitro hydrolysis rates, it was widely accepted that mEH possesses only a minor role in EET hydrolysis in vivo [5]. Thus, while mEH null mice were developed before sEH null mice, few studies have examined the impact of mEH on EET metabolism or EET-mediated physiology in vivo [11, 12]. The recent discovery of a prominent role for mEH in EET metabolism suggests additional studies on the role of mEH in cardiovascular biology are warranted.

mEH-mediated epoxide hydrolysis

P450s and mEH detoxify reactive and hydrophobic compounds to less reactive, hydrophilic compounds that are more readily excreted [1]. That detoxification often involves transformation through highly reactive intermediates suggesting a benefit to tight coordination between P450s and mEH to prevent temporary release of the reactive intermediates [13]. Indeed, both P450s and mEH are integral membrane proteins most commonly found in the endoplasmic reticulum (ER) and are believed to participate

in coupled reactions [14–16]. More recently, close co-localization or direct physical interaction between mEH and P450s has been observed in live cells [7, 17].

The rate of mEH-mediated hydrolysis of xenobiotic epoxides is lower than that of other hydrolases which seems counterintuitive given its important protective role in detoxification. Oesch et al. have proposed that mEH-mediated metabolism occurs via a two-step mechanism. First, mEH rapidly binds the epoxide and isolates reactive epoxides from the system [18]. Second, epoxide hydrolysis and diol release regenerate a free protein. The second step is up to three orders of magnitude slower than epoxide binding [1]. In this model, reactive epoxides are efficiently removed even if the overall rate of hydrolysis is slow, ~ 1 s^{-1} for many epoxides. However, mEH remains a capable detoxification enzyme if mEH protein exists at a concentration that is higher than that of reactive epoxides. mEH represents up to 1% of all microsomal proteins and exists at concentrations up to 50 µM in cells; thus, mEH can overcome its low rate of turnover to readily detoxify reactive xenobiotics that exist at lower concentrations [1, 2]. Additionally, it is possible that in vitro assays underestimate the rate of mEH mediated hydrolysis, as membrane anchoring or physical interaction between mEH and P450s may facilitate coupling of substrate metabolism and/or provide an allosteric enhancement of mEH activity [14, 16].

mEH in xenobiotic metabolism

mEH plays a critical role in the metabolism of both pharmaceuticals and environmental chemicals. mEH typically acts as a phase II enzyme in xenobiotic metabolism following substrate epoxygenation by cytochromes P450s For example, the commonly prescribed anti-epileptic drug phenytoin can be oxidized by cytochromes P450 to a teratogenic arene oxide intermediate that can be detoxified by mEH-mediated hydrolysis [19]. Similarly, one pathway for styrene detoxification involves P450-mediated formation of styrene oxide that is then hydrolyzed by mEH to styrene glycol [20]. The role of mEH is critical in both cases. Inhibition of mEH in pregnant mice treated with phenytoin results in birth defects, while styrene treatment of mEH-null mice causes severe hepatotoxicity [19–21].

Not all the actions of mEH are protective. For example, mEH detoxifies numerous genotoxic and carcinogenic compounds, including naphthalene, benzene, and butadiene [22]; however, mEH bioactivates the genotoxic potential of some polycyclic aromatic hydrocarbons, including benzopyrene or dimethylbenzathracene (DMBA) [12, 23]. Thus, the role mEH in the complex pathobiology of smoking-induced lung cancer, for example, may be multi-faceted.

mEH in epoxy-fatty acid metabolism

For decades, sEH has been considered the predominant enzyme involved in the regulation of EpFA hydrolysis [5]. The importance of mEH in EpFA hydrolysis became apparent only recently in studies using mEH/sEH double null mice. sEH null mice have significant increases in EpFAs and decreases in corresponding diols; however, these changes are most obvious only for its preferred substrates, 14,15-EET and 12,13-EpOME [7, 9]. In contrast, mEH null mice exhibit only minor changes in EET hydrolysis or plasma EET and DHET levels. The role of mEH in EpFA hydrolysis was revealed in mEH/sEH null mice which have nearly complete absence of plasma diols for all the regioisomerjc epoxides derived from AA, EPA, and DHA [7]. Thus, despite large differences in in vitro rates of metabolism of various EpFA regioisomers, mEH and sEH both contribute to hydrolysis of nearly every EpFA examined. The lone exception is 12,13-EpOME, which appears hydrolyzed by sEH but not mEH. Conversely, mEH null mice have increased plasma 19,20-EpDPE compared to wild-type and sEH null mice, which suggests that mEH may play a relatively important in DHA epoxide hydrolysis. Disruption of both mEH and sEH synergistically increase nearly all plasma EpFA levels [7].

The mechanism by which mEH substantially contributes to EpFA hydrolysis is not entirely clear. mEH is a membrane-anchored protein mostly localized in ER membranes adjacent to P450s [17]. One model (Fig. 2) suggests that, despite a low rate of EpFA hydrolysis, mEH is expressed at sufficient levels to hydrolyze epoxides during slow, basal EpFA formation. mEH hydrolysis of EpFAs may occur in a coupled reaction with P450s that directly produces fatty acid diols, similar to that suspected for xenobiotic metabolism of butadiene and styrene [1, 13]. Upon stimulation, the cellular formation of EpFAs may increase to a degree that surpasses the capacity of mEH and thus allows the accumulation of EpFAs. Under these conditions, sEH plays a more prominent role.

The relative rates of sEH to mEH metabolism are less important than their access to substrate. Liver sEH levels have been reported as high as 400 nM and mEH levels may be 10–50 µM, while tissue concentrations of EpFAs are in the low nanomolar range [1, 2, 24]. Thus, most cells have enormous excess sEH and mEH capacity for EpFA hydrolysis. The rate of EpFA hydrolysis by mEH may be slow relative to the excess capacity of sEH, but it is efficient enough to mediate EpFA hydrolysis in vivo under basal conditions. Consequently, the rate of mEH versus sEH metabolism appears less important than the level of expression or access to substrate, as is the case for most hydrolases [24]. Intriguingly, while mEH was considered to play a minor role in EpFA metabolism, it is possible that mEH, localized

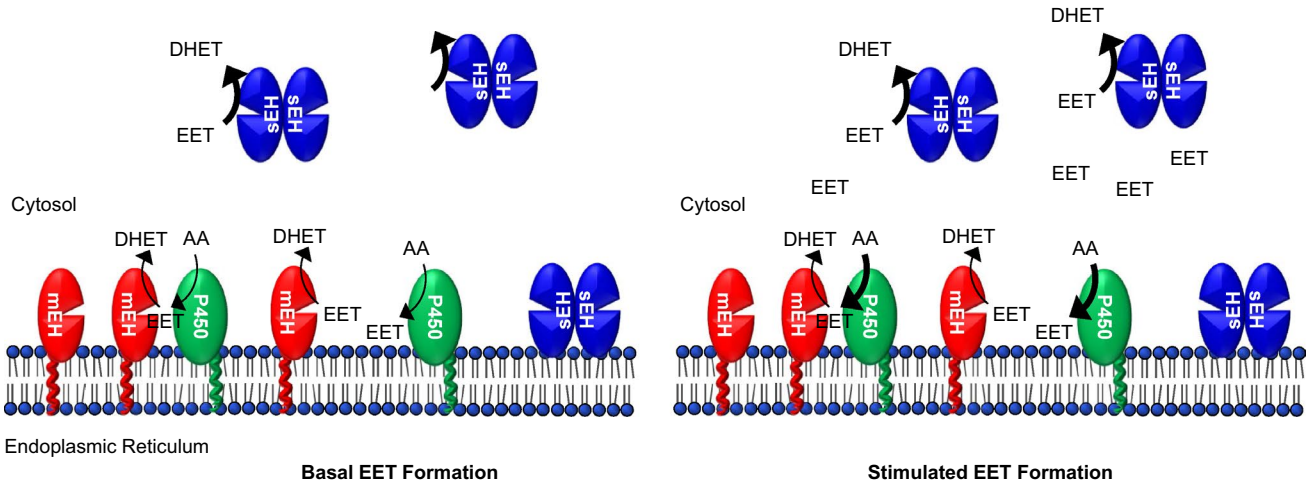

Fig. 2 Contribution of mEH and sEH to EET metabolism. mEH is mostly restricted to membranes of the endoplasmic reticulum, while sEH is found both in both microsomes and cytosol. During basal formation of EETs from AA (left), mEH localization and expression are capable of regulating EET levels in vivo despite its limited catalytic rate. mEH may participate in a coupled reaction with P450s or simply be localized adjacent to EET formation. During stimulated EET formation, P450 formation of high local EET concentrations surpasses the catalytic capacity of mEH. The resulting burst of EETs are ultimately returned to baseline via sEH-mediated hydrolysis to less biologically active DHETs

adjacent to P450s, has first access to substrate and mediates the majority of diol formation during basal EpFA formation [5, 7]. Together, this data also suggests that SNPs that alter expression of mEH or sEH may be biologically more important than SNPs that induce a minor change in the rate of mEH or sEH EpFA hydrolysis.

Combined global deficiency of mEH and sEH is unlikely to occur in nature, as subjects possessing inactivating mutations of both enzymes would be extremely rare. However, the discovery that both mEH and sEH contribute to EpFA hydrolysis may be important in tissues or cells that may be mostly deficient in either of the epoxide hydrolases. For example, the lung, esophagus and brain have very low sEH protein and mRNA levels but have a relative abundance of mEH mRNA and protein (https://www.proteinatlas.org/ENSG00000143819-EPHX1) [5]. Even in tissues with high expression of both sEH and mEH, expression of each hydrolase is compartmentalized. For example, in the liver, both sEH and mEH are abundant in hepatocytes and epithelial cells of the bile ducts; however, only mEH expression is found in vascular endothelium and Kupffer cells [8]. Similarly, in brain, sEH is abundant only in astrocytes and selective neuronal cell populations, whereas mEH is more broadly expressed in neurons as well as smooth muscle, epithelial and endothelial cells [10]. In these sEH-deficient or "sEH-low" cells and tissues, mEH will become the major EpFA hydrolase, and polymorphisms that alter mEH expression and/or activity will have the greatest impact on EpFA hydrolysis and signaling. Thus, while mEH polymorphisms that alter mEH-mediated detoxification of xenobiotics may impact the development of exposure-related inflammation or cancer, these SNPs may also significantly regulate EET inactivation and subsequent effects of EETs on inflammation and tumor progression.

EPHX1 polymorphisms

Two human *EPHX1* SNPs have been most widely studied. The SNP rs1051740 encodes for a T337C missense substitution that changes tyrosine 113 to histidine (Y113H) in mEH proteins. Y113H is most common in individuals of East Asian and European descent (MAF 48% and 30%, respectively) but much lower prevalence in those of African descent (MAF 14%) [3, 25]. The SNP rs2234922 encodes for A416 > G missense substitution that changes histidine 139 to arginine (H139R) in expressed proteins. H139R is most common in individuals of African descent (MAF 35%) but rarer in those of European and East Asian ancestry (MAF 16% and 12%, respectively) [3, 25]. The Y113H polymorphism reduces mEH activity by 39%, while H139R increases its activity by 25% in vitro[26]. Lower and higher activity mEH variants also appear to correlate with styrene and aromatic hydrocarbon metabolism in vivo [27, 28].

mEH regulation of cancer

The role of *EPHX1* polymorphisms in cancer has been extensively reviewed elsewhere [1–3]. Association of *EPHX1* variants with various cancers have been investigated in over 200 published studies [3]. *EPHX1* polymorphisms are most commonly studied for associations with lung, esophagus and colorectal cancers, but have also been examined with prostate, bladder and breast cancers [1–3]. Most studies have reported mixed or inconclusive data. Lung cancer, which is heavily influenced by smoking or environmental exposures, is a strong candidate to be regulated by alterations in mEH-mediated xenobiotic metabolism; however, the low activity Y113H mEH variant has been found to be protective [29, 30] or deleterious [31, 32] depending on the study. Meta analyses suggest that Y113H is either protective or has little effect on lung cancer incidence [33]. In contrast, the high activity H139R mEH variant is associated with increased DNA adduct formation in smokers and is more consistently associated increased lung cancer risk [34, 35]. Overall, these findings suggest that mEH activity modestly exacerbates lung cancer through activation of polycyclic aromatic hydrocarbons as has been observed in animal models. Indeed, mEH null mice are protected against DMBA or benzene carcinogenicity or toxicity [12, 36].

mEH regulation of cardiovascular biology

EpFAs regulate inflammation, cardiovascular function and cancer through a variety of signaling processes reviewed in detail elsewhere [2, 37]. While EETs are believed to act through an as yet unidentified G-Protein-Coupled Receptor, they can also signal through ion channels or nuclear receptors [37]. Regardless of the proximal signaling events, EETs regulate cardiovascular biology in several ways. EETs are considered to be endothelial-derived hyperpolarization factors (EDHFs) which indirectly activate large-conductance calcium-activated potassium (BK_{Ca}) channels to hyperpolarize vascular smooth muscle cells and induce vasodilation [38], activate glycogen synthase kinase 3 beta (GSK-3β) and open ATP-sensitive potassium (K_{ATP}) channels to protect against hypoxia [39], reduce nuclear factor kappa B (NF-kB) activation to attenuate inflammation [40], induce extracellular signal-regulated kinases (ERK) activation to induce endothelial and tumor cell proliferation and migration [41] and synergize with vascular endothelial growth factor (VEGF) to induce angiogenesis [42].

Few animal models have investigated the role of mEH in cardiovascular biology, though several studies indicate that mEH metabolism of EpFAs to less biologically active diols may regulate vascular function. In brain, EETs can directly dilate cerebral arteries [43]. Substitution of glutamic acid 404 to aspartic acid (E404D) increases the Vmax of mEH toward 9,10-epoxystearic acid by 40-fold [44]. Brain microsomes from mice that express only the E404D mEH variant display enhanced EET hydrolysis in vitro and increased plasma DHET:EET ratios which reflect an increase in EET hydrolysis in vivo. Moreover, E404D mice display reduced vasodilatory capacity to enhance cerebral blood flow in both hippocampus and cortex [45]. Thus, mEH-mediated hydrolysis of EpFAs to less vasodilatory diols can significantly regulate of blood flow in brain, and may regulate vasodilation in other tissues, such as the liver, where endothelial mEH expression is high and endothelial sEH expression is low [8, 45].

mEH also metabolizes EpFAs to regulate cardiac function. Hearts abundantly express both mEH and sEH[7]. sEH inhibition, sEH genetic disruption, CYP2J2 overexpression, or exogenous EET treatment have all been previously shown to improve cardiac recovery after ischemia [39, 46, 47]. EET-mediated activation of mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), GSK-3β, and/or K_{ATP} channels protect mitochondria against damage during post-ischemic reperfusion [48, 49]. While mEH disruption doesn't significantly alter cardiac EpFA metabolism or recovery after ischemia, combined disruption of mEH and sEH abolishes EET hydrolysis and synergistically improves cardiac recovery after ischemia [7]. Thus, mEH plays a secondary, but important role in the regulation of EpFAs and post-ischemic contractile function in the heart.

EPHX1 polymorphisms that increase mEH activity are associated with an increased risk of preeclampsia, a severe pregnancy complication characterized by hypertension and proteinuria [50–53]. The etiology of preeclampsia and its regulation by EpFAs are unclear. Moreover, the correlation of EPHX1 polymorphisms with preeclampsia have not been associated with alterations in metabolism of any particular mEH substrate class [3]. Interestingly, polymorphisms in other genes involved in EpFA metabolism have also been recently associated with preeclampsia. Polymorphisms that increase sEH expression or activity, which would lower EpFA levels, are positively associated to preeclampsia [54, 55]. In contrast, actual levels of EpFAs are increased in placenta, plasma and urine of preeclamptic women, though this may reflect compensatory induction of cytochromes P450 and EpFA formation in response to the hypertension [56–58]. Increased mEH-mediated hydrolysis of EpFAs could contribute to the induction of preeclampsia though dysregulation of angiogenesis required for normal placental vascular development. Alternatively, increased hydrolysis of vasodilatory EpFAs could exacerbate end-stage hypertension in preeclampsia.

mEH-mediated hydrolysis of EpFAs may also contribute to disease states commonly associated with its role in

xenobiotic metabolism. For example, sEH null mice have increased EpFAs, angiogenesis, primary tumor growth and metastasis in some cancer models [42]. Interestingly, sEH protein levels are often downregulated in tumor endothelium and cancer cells [42, 59]. While *EPHX1* polymorphisms may alter the risk of cancer initiation after exposure to environmental agents, *EPHX1* polymorphisms may subsequently alter hydrolysis of EpFAs that regulate angiogenesis and/or cell proliferation to influence the development of tumors or metastasis [3, 60]. Unlike sEH, mEH expression appears to be increased in several cancer cell types, which suggests that mEH-mediated EpFA hydrolysis likely acts to counter tumor growth and metastasis [61, 62].

mEH is broadly expressed in the brain and likely plays a neuroprotective role in metabolism of certain xenobiotics that can cross the blood–brain barrier; however, mEH may also regulate inflammatory processes during neurodegenerative diseases [10]. Several studies link sEH to neurodegenerative disorders. For example, sEH expression and activity are increased in subjects with cognitive decline and sEH null mice are protected against Alzheimer's disease progression [63, 64]. These studies suggest that sEH-mediated hydrolysis may contribute to neurodegeneration though alteration in anti-inflammatory EpFA levels. Few studies have examined the role of mEH in neurodegenerative diseases, though mEH expression is elevated in subjects with Alzheimer's disease [65]. Given the more pronounced role of mEH in EpFA metabolism in the brain, *EPHX1* polymorphisms and/or mEH expression may also significantly regulate the anti-inflammatory and neuroprotective effects of EpFAs.

mEH was previously presumed to play a major role in detoxification but a minor role in EpFA metabolism; however, recent evidence suggests that it has a broader substrate range and exerts a significant impact on EpFA levels in vivo. The development of pharmacokinetically practical mEH inhibitors has lagged behind that of sEH inhibitors. Moreover, sEH inhibitors are often screened to avoid off-target effects on mEH [66]. Given its importance to xenobiotic detoxification, long term use of mEH inhibitors is likely unwise; however acute mEH inhibition may be beneficial in some settings, such as myocardial infarction, stroke, preeclampsia and vascular inflammation. Uncovering the role of mEH in EpFA metabolism opened the door to an entirely new perspective on the role of mEH and *EPHX1* polymorphisms in cardiovascular disease pathogenesis.

Acknowledgements This work was supported by the Division of Intramural Research, National Institute of Environmental Health Sciences, NIH (Z01 ES025034 to D.C.Z.)

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

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