13 Epoxide Hydrolases

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13.1 SUMMARY

Epoxides are three-membered carbon–oxygen compounds that typically arise from the metabolism of endogenous and xenobiotic compounds via chemical and enzymatic oxidation processes. Many different oxygenase enzymes are capable of forming epoxide derivates, from either arene or alkene substrate; however, the cytochrome P450 monooxygenases are principal contributors to their generation. Certain epoxides are highly electrophilic and chemically reactive, and they have been implicated as initiators of various cellular toxicities, including the formation of DNA mutations and ultimately, cancers. Therefore, it is of vital importance for the organism to regulate levels of these reactive species. A single mammalian enzyme, microsomal epoxide hydrolase (EPHX1, mEH), functions as a predominant pathway responsible for detoxification of xenobiotic epoxides, as well as bioactivation pathway for certain xenobiotic epoxides. In contrast, another important epoxide hydrolase, soluble epoxide hydrolase (EPHX2, sEH), has more recently been established as a mediator of epoxide hydrolysis of several endogenously derived epoxyeicosatrienoic acids (EETs), intermediates resulting from arachidonic acid metabolism. In this respect, EPHX2 is now characterized as an important regulator of physiological processes, such as blood pressure and inflammation, and has emerged as a promising therapeutic target for pharmacological intervention. This chapter reviews the biochemistry, biological regulation, genetics, and pharmacological relevance of these important enzyme systems.

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13.2 INTRODUCTION TO EPOXIDE HYDROLASES

A wide variety of endogenously and exogenously derived lipophilic aromatic and aliphatic substances are metabolized initially through phase I biotransformation pathways, perhaps most notably by the cytochrome P450 monooxygenase enzymes (CYPs) [1]. The resulting oxidative metabolites include epoxide-containing intermediates that are subject to further metabolism by both phase I and phase II biotransformation pathways. Cells have developed the capacity to metabolize chemical epoxides through several pathways. Prominent among these are the epoxide hydrolases, including EPHX1 (EC 3.3.2.9) and EPHX2 (EC 3.3.2.10). The epoxide hydrolases belong to a subcategory of a broad group of hydrolytic enzymes that include esterases, proteases, dehalogenases, and lipases [2,3]. Epoxide hydrolases catalyze the hydrolysis of epoxides to their corresponding dihydrodiol (glycol) products. The hydration reactions often lead to changes in the biological functionality and/or the chemical reactivity of the resulting metabolite. Simple epoxides are hydrated to their corresponding vicinal dihydrodiols and arene oxides to *trans*-dihydrodiols. Although certain epoxides are relatively stable, for example, the pharmaceutical metabolite carbamazepine (CBZ) epoxide, other chemical epoxides are highly electrophilic and may react with cellular nucleophiles, such as DNA, RNA, and proteins [4]. With respect to xenobiotics, epoxide hydrolase metabolism results principally in detoxification. Importantly however, in certain instances, bioactivated toxic intermediates of xenobiotic substances may also be generated. Frequently, reactive and unstable epoxide metabolites, such as the bay-region and fjord-region diol epoxide intermediates arising from polyaromatic hydrocarbon (PAH) metabolism, have been identified as ultimate cytotoxic and carcinogenic reaction products [5,6]. The overall balance of xenobiotic metabolism between bioactivation and detoxification pathways catalyzed by EPHX1 determines the pharmacokinetic disposition and the ultimate fate of reactive intermediates within target cells. In contrast to EPHX1, the substrate specificity of EPHX2 is directed toward endogenously derived and generated epoxides, in particular, those resulting from the arachidonic acid metabolism cascade [3]. These intermediates function to regulate several important physiological processes, such as vascular reactivity and inflammatory responses.

13.2.1 Types and General Reactions of the Epoxide Hydrolases

Epoxide hydrolases are members of the α/β-hydrolase fold superfamily. The α/β-hydrolase fold domain structure α/β domain (ABD) is common to a number of hydrolytic enzymes of widely differing phylogenetic origins and catalytic functions. The α/β-hydrolase fold is characterized by a β-sheet core of five to eight strands linked by α-helices to form a α/β/α sandwich structure [7]. The enzymatic activities contributed by members of this superfamily include esterase, lipase, and hydrolase functionalities [7,8]. Members of other gene families may also catalyze hydrolase activities, described briefly below.

Several types of mammalian epoxide-hydrolyzing enzymes have been characterized. These include (i) a microsomal enzyme specific for cholesterol 5,6-epoxide hydrolysis [9] that is little studied but distinct from the ABD-containing hydrolases otherwise discussed in this chapter; (ii) a membrane-associated hepoxilin A3 hydrolase, also likely distinct from the ABD domain structure, participates in arachidonic acid metabolism in the central nervous system and vascular cells [10]; (iii) a cytosolic leukotriene

A4 (LTA4) hydrolase that is quite distinct from the ABD protein family, as it possesses a zinc-binding domain and aminopeptidase activity, which mediates leukotriene metabolism and is consequently implicated in inflammatory and allergic responses [11,12]; (iv) an mEH (EPHX1) that is active in the metabolism of certain pharmaceuticals as well as a broad range of xenobiotic compounds, such as the mutagenic PAH-derived epoxides [2]; (v) an sEH (EPHX2) that is active in the metabolism of arachidonic-acid-derived epoxides and other endogenous epoxides [13]; and, the two more recently identified epoxide hydrolases, (vi) EPHX3 and (vii) EPHX4. EPHX3, also termed *ABHD9*, is a predicted α/β-hydrolase fold superfamily member that was identified in a genomics screen for DNA methylation markers associated with prostate cancer prognosis [14]. EPXH4 was identified by genomic-sequencing-based coding queries as a putative α/β-hydrolase-domain-containing protein, potentially associated, along with many other polymorphic markers, with altered response to hepatitis B vaccination [15]. Little other functional data are available describing EPHX3 or EPHX4, although apparent homologs of these hydrolases have been identified in *Caenorhabditis elegans.* EPHX3 and EPHX4 appear structurally much more closely related to EPHX2 than to EPXH1 and therefore may have a role in generating lipid-signaling intermediates [16].

The EPHX1 and EPHX2 members of the α/β-hydrolase fold family of proteins are two of the most well-investigated mammalian epoxide hydrolases. EPHX1 and EPHX2 are distinguished on their subcellular distributions, typically associated with microsomal and cytosolic distributions, respectively, but perhaps, more importantly, they possess highly distinctive substrate specificities and physiological roles. It is likely that two independent forces, namely, cytoprotection and cellular signaling, drove the evolution of EPHX1 and EPHX2, respectively [13]. Of the various epoxide hydrolases, that is, EPHX1, is *the only* hydrolase known to possess enzymatic activity toward xenobiotic-derived epoxides [13], and therefore, it is a principal focus in this chapter. However, EPHX2 is rapidly generating intense interest as a pharmaceutical target, with potential application in drug therapies aimed at treatment of inflammatory, cardiovascular, and other diseases. Consequently, this chapter reviews both these hydrolases and includes discussion of their respective functional, regulatory, and genetic features.

13.3 MICROSOMAL EPOXIDE HYDROLASE (EPHX1)

13.3.1 Introduction

As the earliest known epoxide hydrolase, EPHX1 plays a critical role in the metabolism of many xenobiotic compounds. The name of this epoxide hydrolase is derived from its primary cellular localization in the endoplasmic reticulum, that is, the microsomal environment. mEH appears unique among all the epoxide hydrolases for its exclusive role in the metabolism of xenobiotic-derived epoxides. EPHX1 is capable of hydrolyzing epoxides derived from xenobiotics of highly diverse structures, and the enzyme is especially capable of accepting substrates with bulky configurations, such as arene oxides [2,17]. The diol products of EPHX1 hydrolysis typically are less reactive chemical species relative to the parent compound, and the enzyme is therefore considered to generally foster detoxification. However, in the case of arene oxide metabolism, subsequent CYP-mediated ring oxidation of the hydrolyzed metabolite can lead to the

formation of vicinal diol epoxide species that may possess extremely high chemical reactivity [6,18].

The catalytic mechanism of EPHX1 involves two steps and is similar to that described for EPHX2 [19,20]. In this mechanism, first there is attack of a nucleophilic aspartic acid on the oxirane ring to yield an alkyl enzyme intermediate, and then subsequent hydrolysis of the intermediate by water. On the basis of sequence alignment analysis, the catalytic triad of EPHX1 was identified as consisting of Asp226, His431, and Glu404 [20,21]. Subsequently, site-directed mutagenesis experiments in a rodent model confirmed these catalytic amino acids as critical for EPHX1 enzymatic activity [3]. The human EPHX1 enzyme requires no cofactors for activity.

The human EPHX1 gene exists as a single copy and is located on chromosome 1, specifically at position 1q42.1 [22,23]. The coding region of the EPHX1 gene locus spans ~20 kb. The EPHX1 gene product exists as a monomeric protein of ~49 kDa and is composed of 455 amino acids [23]. Human EPHX1 is expressed in all tissues thus far examined, with highest levels in the liver, lower yet comparable levels in kidney and ovary, and lower levels in testis, lung, adrenal glands, and lymphocytes [13,24,25]. Developmentally, EPHX1 expression in human fetal tissues is relatively low during early gestation but increases as gestation progresses [26]. Research conducted with experimental rodent models indicates that the EPHX1 gene is responsive to chemical treatments [27–29]; however, evidence obtained from primary hepatocytes indicates that EPHX1 is only modestly transcriptional responsive in humans [30]. Early studies, cited above, drew attention to associations between EPHX1 and cancer. In humans, EPHX1 protein has been consistently identified in tumors from a variety of organs [31–35], fueling speculation that expression of this enzyme may play a molecular role in malignancy. Importantly, experiments employing null mice have demonstrated that EPHX1 expression is a critical determinant in PAH-induced carcinogenesis [36].

13.3.2 Biological Function of EPHX1

Over the last few decades, EPHX1 has been recognized primarily for its role in xenobiotic metabolism. However, several intriguing observations also suggest that EPHX1 may play a role in modulating physiological functions. For example, several lines of evidence indicate that EPHX1 may play a role in steroid synthesis and/or metabolism [13]. For example, estroxide and androstene oxide are good EPHX1 substrates [37,38]. Consistent with this theme, an early study identified EPHX1 as a subunit of the antiestrogen binding site [39]. Furthermore, EPHX1 is relatively highly expressed in ovaries [40,41], especially in follicle cells [42]. One hypothesis is that EPHX1 may be important for cellular protection against reactive metabolites of endogenous compounds, such as epoxy steroids. Human EPHX1 is expressed and developmentally regulated in fetal tissues [26], suggesting that it may be important for protection from toxic epoxide intermediates during embryonic and fetal development. In this light, a recent report indicated that oviductal EPHX1 expression is upregulated during the process of mouse embryogenesis and that increased oviductal EPHX1 may help reduce levels of reactive oxygen species [43]. These functions may represent protective effects of EPXH1, potentially enhancing mouse embryo developmental processes.

In addition to a potential role in steroid homeostasis, another study suggested that EPHX1 is a functional component of the vitamin K_1–oxide reductase complex in rat liver microsomes [44]. Also, a role for EPHX1 in bile acid homeostasis has been

suggested, as EPHX1 was identified by one group of investigators as part of a multiprotein transport system that is responsible for sodium-dependent bile acid uptake in liver [45–48]. To further this view, several reports have indicated that EPHX1 is expressed at the hepatocyte plasma membrane and in the endoplasmic reticulum where it can exist in two topological orientations and that bile acid uptake appears dependent on EPHX1 expression at the plasma membrane [48]. However, the specific role and relative importance of EPHX1 in the physiologic function of bile acid transport is controversial, in that another study was unable to reproduce these purported activities of EPHX1 [49]. Possibly, development and use of potent and selective EPHX1 inhibitors may help to address the still open questions as to a potential role of EPHX1 in bile acid transport and/or vitamin K reductase participation.

As EPHX1 is expressed ubiquitously in many species, including mammalian, plant, and single cell organisms [50], and several intriguing lines of evidence for its endogenous functionality have been advanced, it seems logical to postulate an important physiological role for its activity. However, it should be noted that an EPHX1 null mouse model has been established [36]. These null mice appear healthy and reproduce with normal efficiencies. Thus, lack of EPHX1 expression in the mouse does not appear to be physiologically detrimental, presenting somewhat of a puzzling dilemma regarding the potential endogenous role of the enzyme in mammalian systems.

13.3.3 Substrates and Inhibitors

EPHX1 exhibits a large range of substrate specificity, and most epoxide intermediates are generated *in situ* by phase I oxidation reactions [51]. The substrates of EPHX1 broadly range from aliphatic epoxides to polyaromatic oxides. The subsections below review a number of chemically derived, environmentally relevant, and pharmaceutical substrates for the enzyme, together with the biological implications of these reactions.

13.3.3.1 Environmental/Xenobiotic. EPHX1 has been recognized as an important contributor to the metabolism of xenobiotic epoxides, including numerous environmental contaminates. Historically, several different substrate probes for EPHX1 have been employed for routine assessments of enzymatic activity in biochemical reactions. These include benzo[*a*]pyrene (B[*a*]P)-4,5-oxide [52,53], *cis*-stilbene oxide [54], styrene oxide [55], CBZ-10,11-epoxide [56,57], and naphthalene-1,2-oxide [57,58]. Fluorescent substrates for EPHX1 have more recently been developed. The highest activity for both rat and human EPHX1 was obtained with the fluorescent substrate cyano(6-methoxy-naphthalen-2-yl)methyl glycidyl carbonate [59]; however, this compound cannot be used with crude enzymatic preparations due to interfering reactions.

Common environmental toxins that generate epoxide intermediates metabolized by EPHX1 include 1,3-butadiene [60,61], styrene [62], and benzene [63]. Illustrations of the structural features of these substrates and associated reactions are shown in Fig. 13.1. In addition, several other xenobiotic compounds are metabolized to epoxide intermediates, such as aflatoxin B_1 [64], the nitropyrenes [65,66], and the PAHs, including chrysene, naphthalene, anthracene, and B[*a*]P [67,68].

As a case in point, PAHs are products of incomplete combustion of organic matter and are widespread environmental contaminants found in automobile exhaust, cigarette smoke, air, water, food, and soil. PAHs are considered procarcinogens because they

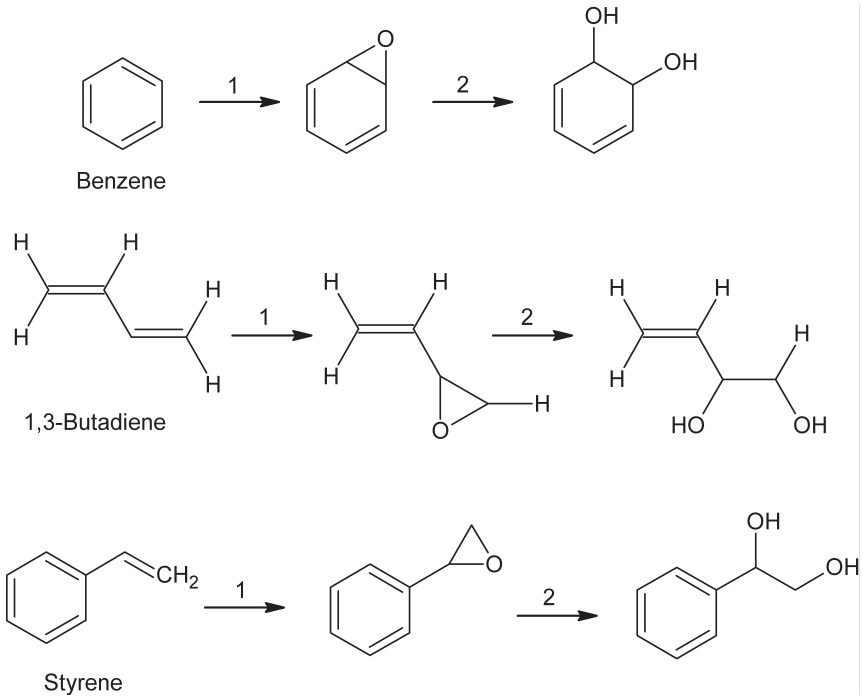

Figure 13.1 Common xenobiotic substrates of EPHX1 and their general reaction schemes. Step one is commonly mediated via cytochrome P450, while step 2 is mediated via EPHX1.

require metabolic activation to electrophilic reactive metabolites to exert their mutagenic and tumorigenic activities. Three principal pathways of metabolic activation have been proposed for the activation of the PAHs. These involve (i) the formation of diol epoxides by several CYPs, where CYP1A1, CYP1B1, and others can play a major role [69]; (ii) the formation of radical cations catalyzed by peroxidases [70]; and (iii) the formation of reactive intermediates catalyzed by dehydrogenases and other reductases [71,72]. However, the biological effects of a number of PAHs, including their mutagenicity and carcinogenicity, are largely dependent on enzymatic activation to dihydrodiol epoxide intermediates [73], which are formed by a three-step process: initial epoxidation by the CYPs, subsequent EPHX1-mediated hydrolysis to the *trans*-dihydrodiol, followed by a second epoxidation at the adjacent double bond [74]. The critical nature of EPHX1 bioactivation in PAH-induced carcinogenesis is further demonstrated in EPHX1 null mice, which are completely resistant to the tumorigenic effects of the PAH dimethylbenz[*a*]anthracene in a complete carcinogenesis assay [36].

The mutagenic and carcinogenic potency of the PAHs appears largely dependent on their structural features. As indicated previously, K-region PAH oxides, such as the B[*a*]P-4,5-oxide, are often used as model substrates for EPHX1 activity. Although mutagenic, these derivatives are not the principal determinants of PAH-induced carcinogenesis. Rather, it is the terminal ring PAH oxides, away from the respective bay or fjord region and when hydrolyzed by epoxide hydrolase, that lead to production of the proximate PAH carcinogen, the *trans*-dihydrodiol, which in turn is metabolized to the ultimate carcinogen, the diol epoxide. The strong steric interactions of the fjord-region

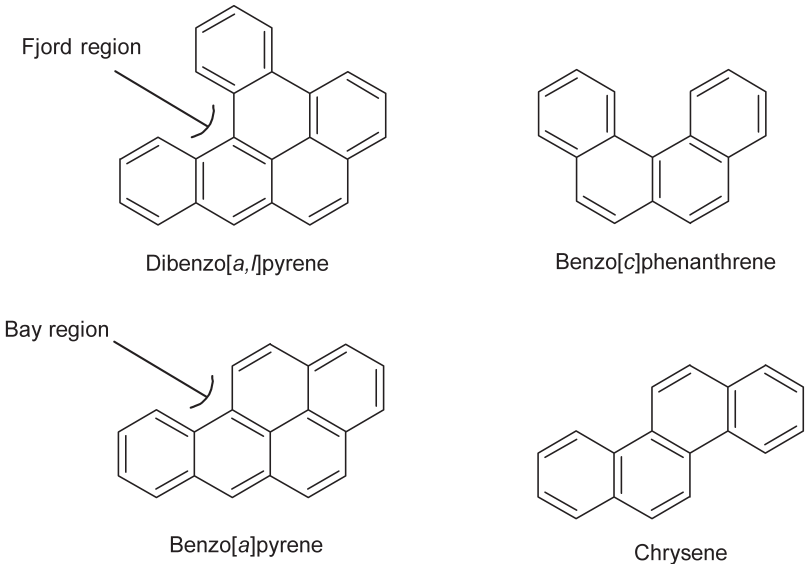

Figure 13.2 Examples of polyaromatic hydrocarbons illustrating both bay- and fjord-region centers that are subject to epoxidation reactions by cytochrome P450s and subsequent hydrolysis by EPHX1.

force the molecule to distort from planarity. EPHX1 contributes both to the formation of highly reactive and mutagenic bay-region (planar PAHs) and fjord-region (nonplanar PAHs) diol epoxide intermediates [6,75,76], with the corresponding mutagenic and tumorigenic potencies of these substances largely dependent on their structural features. In particular, diol epoxides derived from PAHs that possess a fjord region exhibit substantially more potent carcinogenic activities than those derived from bay-region PAHs [6]. Examples of both bay-region and fjord-region PAHs are shown in Fig. 13.2. The diol epoxides thus generated react readily with mutational hot spots in DNA to form stable adducts both *in vitro* and *in vivo* [77,78]. If these adducts are not repaired, they result in misreplication and mutagenesis [77,78]. The fjord-region, nonplanar diol epoxides, such as dibenzo[*a*, *l*]pyrene, react more favorably with deoxyadenosine (dA) than deoxyguanosine (dG) in DNA and fjord-region-modified DNA adducts are more difficult to repair than a bay-region adduct [79–82]. Given this backdrop, it is of interest that several epidemiologic investigations have now implicated human EPHX1 gene-coding-region polymorphisms as a risk modifier for lung cancer incidence [83].

13.3.3.2 Pharmacological. Although many pharmaceuticals undergo oxidative metabolism, principally in the liver but also in other organs, and the CYPs play prominent roles in these initial enzymatic reactions, it is perhaps surprising that not many known pharmaceutical agents undergo sequential enzymatic processing by EPHX1. In part, this is likely due to the extensive preclinical testing that developmental drug candidates are subjected to, and it is logical to assume that compounds exhibiting an avid potential to be metabolized into reactive epoxides are typically eliminated from further development in early toxicity screens. However,

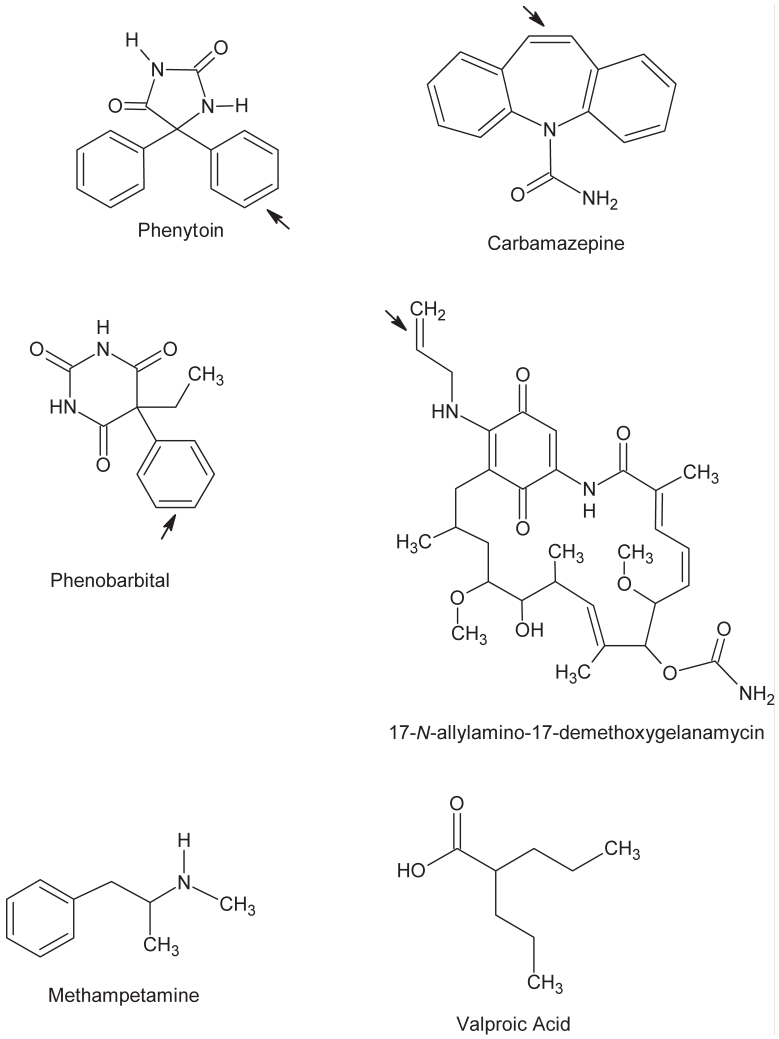

Figure 13.3 Examples of anticonvulsant medications and other pharmacological agents that are implicated in EPHX1 metabolism schemes. Arrows represent epoxidation reaction sites.

EPHX1 has been implicated in the metabolism of several categories of drugs, including commonly used anticonvulsant, psychostimulant, and anticancer drugs.

Historically, aromatic anticonvulsant drugs, such as phenobarbital (PB), diphenylhydantoin (phenytoin), and CBZ, have been widely employed in the treatment of seizure disorders and are the most readily recognized as having interactions with epoxide hydrolase [84]. Figure 13.3 illustrates chemical structures of selected anticonvulsant agents that are metabolized either to epoxide intermediates, which are in turn substrates for EPHX1, or pharmacological structures, which are otherwise known to affect the metabolism of the epoxide hydrolase pathway. Biotransformation pathways for these drugs typically involve initial phase I metabolism by various CYPs that can then result in the generation of arene oxides, which are substrates for EPHX1. Idiosyncratic

adverse drug reactions (ADRs) can occur following treatment with certain anticonvulsants, and in several early studies, polymorphism in EPHX1 expression was implicated as a risk factor for this response [85,86].

For example, phenytoin is an older anticonvulsant medication that has a widespread usage. It is likely metabolized, in part, through an arene oxide intermediate [87]. Toxicity concerns surrounding its use have been frequently raised, and certain evidence implicates EHPX1 in this respect. For example, a case study published in 1988 suggested the protective role for EPHX1 in anticonvulsant hypersensitive syndrome, indicating that patients susceptible to anticonvulsant toxicity had decreased epoxide metabolism activity [88]. In one instance of ADRs, strikingly reduced levels of mEH RNA were demonstrated in an affected patient [89]. The teratogenic risk of fetal hydantoin syndrome, related to exposure to phenytoin, was also reported as increased in a study in subjects with low EPHX1 enzymatic activity [90]. However, subsequent studies that specifically examined the association of EPHX1 amino acid polymorphisms with susceptibility to ADRs found no apparent association [91,92]. It should be noted that the population sizes evaluated in these latter studies were small and additional EPHX1 polymorphisms, for example, those residing in the respective promoter regions of the gene have now been identified [93,94] but have not yet been assessed as potential risk contributors in these respects.

Given the long-standing use of PB for the effective treatment of convulsant disorders, concerns with its use have also arisen. The metabolism of this agent is also projected to proceed, in part, through an arene oxide intermediate [95]. Although PB is a liver-tumor-promoting agent in rodents, epidemiological studies in PB-exposed populations have not uncovered enhanced risk to liver cancer in humans, even following its long-term use [96,97]. Therefore, the larger concern with the use of PB is likely due to its induction effects on various biotransformation pathways [98] that may in turn lead to ADRs or drug–drug interactions. However, the question of whether PB may induce epoxide hydrolase activities in humans is open. In studies conducted using primary hepatocyte cultures from human donors, this agent appears to only modestly effect EPHX1 levels [30], but in general, the issue of interindividual variability in response to chemical inducers certainly adds complexity to these issues [99].

CBZ is another older-generation therapeutic for the treatment of epileptic disorders and has historically been one of the most widely prescribed frontline medications for this syndrome [100]. The principal bioactive metabolite of this compound is the CBZ-10,11-epoxide derivative, an epoxide that is relatively stable and readily detected in serum [100,101]. CBZ-10,11-epoxide is a substrate for EPHX1. Using genetic haplotype-mapping approaches, Japanese investigators reported apparent correlation with EPHX1 coding region genotype status and intrinsic clearance measures of CBZ-10,11-epoxide [101]. More recently, results of independent studies also suggested that the genetic status of EPHX1 may be a useful predictor of CBZ maintenance doses in prescribed populations [102]. Furthermore, it has been reported that valproic acid, another anticonvulsant medication, potentially inhibits EPHX1 activity, thereby altering steady-state levels of CBZ-11,12-oxide in cotreated patients [103]. However, results of other investigations indicate that the glutathione transferase system is likely involved as well in determining the disposition of CBZ-11,12-oxide serum levels [104].

Methamphetamine hydrochloride (METH) is a highly addictive and popular illicit drug that is neurotoxic and presents an addiction that is very difficult to treat. The biotransformation of METH has been investigated. On smoking, pyrolysis products are

derived that include *trans*-phenylpropylene, a product that is likely to undergo oxidation metabolism by the CYPs, leading to a potentially reactive and neurotoxic epoxide intermediate [105]. Interestingly, experimental evidence indicates that this neurotoxic epoxide intermediate is indeed a substrate of EPHX1 [105]. Using an EPHX1 knockout mouse model, a recent report identified that EPHX1 expression plays an important role in detoxifying the reactive epoxide intermediates of METH and in attenuating METH drug addiction [106]. This finding offers a novel therapeutic approach for potential prevention or attenuation of the long-term consequences of METH use disorders in humans.

17-(Allylamino)-17-demethoxygeldanamycin (17AAG) is a potent antiproliferative drug that has been in clinical trials for treatment of chronic lymphocytic leukemia as well as kidney tumors, especially in young patients [107]. Its mechanism of action is through targeting of Hsp90-associated activities and disruption of protein-tethering interactions. 17AAG is extensively metabolized; a diol metabolite is among the major products detected. EPHX1 is likely involved in the production of the diol metabolite, subsequent to hydrolysis of an epoxide intermediate formed on the allyl side chain of 17AAG [108]

13.3.3.3 Inhibitors. In early studies, several epoxide-containing compounds were discovered as EPHX1 inhibitors. For example, 1,1,1-trichloropropene-2,3-oxide [109,110] and cyclohexene oxide [111,112] have been widely used as EPHX1 inhibitors. In addition, other investigators have described primary ureas, amides, and amines [113,114], as well as heavy metals such as divalent mercury and zinc [115], as EPHX1 inhibitors. More recently, Hammock and colleagues screened a series of amides and identified 2-nonylsulfanyl-propionamide as a competitive inhibitor of EPHX1 enzymatic activity, exhibiting a K_I of 72 nM, thus establishing this agent as the most potent inhibitor identified to date. These agents represent useful *in vitro* tools for assessing the involvement of EPXH1 activity in the characterization of chemical and pharmaceutical biotransformations.

13.3.4 Genetic Polymorphisms

Human pharmaceutical and xenobiotic metabolisms are subject to large interindividual differences [116,117]. A variety of parameters are known to contribute to this phenomenon, and genetic constitution is likely of major importance [118]. In addition to the inherited differences in chemical metabolism's capacity and specificity, additional contributors to interindividual differences in risk of toxicity resulting from xenobiotic exposure include age, gender, disease states, tissue differences, and environmental influences, such as diet, smoking habits, occupational exposure to chemicals, and concurrent exposure to other drugs [117,119]. Despite the present era of genomics, achieving a detailed comprehension of the interplay of such variables with man's inherent genetics remains a formidable challenge for the foreseeable future. The ability to predict potential hazards resulting from human exposure to pharmaceuticals as well as other foreign chemicals requires that we elucidate the genetic and regulatory factors that contribute importantly to the risk scenarios underlying human variability in biotransformation and xenobiotic disposition.

Large interindividual variations in EPHX1 activities have been described in human tissues. For example, EPHX1 activity was reported to vary from 8-fold to up to 63-fold

in panels of human liver samples [53,120,121]. After the full-length human EPHX1 DNA sequence was cloned and compared, two nonsynonymous amino acid changes were identified in the EPHX1 coding region. The polymorphism in exon 3 corresponds to amino acid position 113 and results in Tyr (Y) to His (H) substitution (rs1051740). An exon 4 polymorphism at position 139 (rs2234922) encodes a His (H) to Arg (R) substitution. For the polymorphism at position 113, 7.6% individuals were observed with homozygous His, and the frequency of 139 A/A is 4.6%. The distribution of the two alleles follows Hardy–Weinberg equilibrium [2,122]. There are other nonsynonymous polymorphisms reported or listed in the NCBI dbSNP database, but to the authors' knowledge, these polymorphisms either have not been validated or have only been identified at a very low allelic frequency. Consequently, the 113Y/H and 139H/R polymorphisms are recognized currently as the most common EPHX1 amino acid variants in the human population. As many commonly encountered environmental chemicals undergo epoxide metabolism, there is an interest in assessing genetic variation in EPHX1 as a potential risk modulator for other chemicals.

Since the initial identification of these two primary EPHX1 coding polymorphisms, a surprisingly large number of epidemiologic investigations have examined the potential association of disease incidence with EPHX1 genotype. An early review of selected molecular epidemiological investigations examining associations of EPHX1 genotype with cancer susceptibility has been published [123]. Perhaps, the most consistent associations implicate EPHX1 coding region polymorphisms as a risk factor for lung cancer [124–130]. In 2002, Lee *et al*. [128] conducted a meta-analysis of seven previously published studies and reported a significant decrease in lung cancer risk (OR = 0.70, 95% CI = 0.51–0.96) associated with the "low activity" exon 3 H113 homozygous EPHX1 genotype, after adjustment for age, sex, smoking, and center. In 2006, a human genome epidemiology (HuGE) review and meta-analysis of 13 case-control studies was published [83], similarly concluding that the low activity variant H113 genotype of EPHX1 was associated with decreased risk of lung cancer (OR = 0.65; 95% CI = 0.44–0.96). In contrast, other reports have indicated an increased risk of chronic obstructive pulmonary disease (COPD) [131,132] and emphysema [132] with the H113 genotype, despite both diseases being linked to smoking behaviors. Another report has indicated a protective association of the Y113H genotype with COPD [133]. The paradoxical results may perhaps be explained if the various EPHX1 allelic proteins exhibited differential activities on specific substrates involved in diverse disease etiologies. Disease associations with EPHX1 polymorphism at the exon 4/Y139R position have also been reported [134–136]. The mechanistic basis for these associations in relation to the enzymatic function of the xenobiotic hydrolase remains unknown.

Genetic differences in EPHX1 also have been reported as a potential risk modulator of toxicity caused by styrene [137] and butadiene [138,139] exposure in human worker populations, although other studies have not confirmed such associations [120,140]. Interestingly, bioactivation of aflatoxin B_1 by cytochrome P450 enzymes generates a highly reactive epoxide metabolite, and incubation with the EPHX1 enzyme is reported to reduce the aflatoxin's carcinogenic effect [64]. In these respects, an initial study indicated a risk association with hepatocellular carcinoma and EPHX1 genotype [141]; however, subsequent analyses were also not able to confirm this association [142].

It should be noted that many of the published EPHX1 molecular epidemiologic reports frequently refer to quantitative functional descriptors for the respective EPHX1

genotypes, indicating, for example, "the low activity allele" or "the high activity genotype." However, the relative enzymatic contribution of the commonly studied EPHX1 Y113H and H139R polymorphisms has been examined functionally to only a limited extent, and only modest support for any enzymatic/catalytic difference among the EPHX1 variants exists. For example, using the K-region, B[*a*]P-4,5-epoxide as substrate, the four potential allelic variants of EPHX1 were evaluated for relative functional activities using sources of EPHX1 protein variously derived from transfected COS-1 cells, baculovirus-infected Sf9 cells, and genotyped samples of human liver microsomes [122,143]. Following normalization to immunoreactive levels of EPHX1 protein, only minimal differences in enzymatic specific activities were apparent among the variant isoforms. Similar results were reported using *cis*-stilbene oxide, another model substrate for EPHX1 [143]. Other investigators also examined the enzymatic capacity of the respective EPHX1 variants and failed to discern a correlation between EPHX1 polymorphism and enzymatic activity [57]. Therefore, despite the many epidemiologic studies that have used descriptors such as "low activity allele" or "high activity allele" in an attempt to explain the functional basis of their results, the underlying basis for the EPHX1 activity claim is not yet well substantiated. Given the important role of EPHX1 in the biotransformation of reactive xenobiotic epoxides and the apparent wealth of epidemiological association of genetically encoded differences in EPHX1 protein structure with the incidence of various diseases, the exact functional impact of EPHX1 polymorphism requires more rigorous study. Moreover, the interaction of EPHX1 with other metabolizing enzymes, such as the glutathione *S*-transferases and *N*-acetyltransferases, may additionally contribute to modulate the association with lung cancer [144]. Since the EPHX1 and EPHX2 metabolic pathways are each intimately connected to cytochrome P450-mediated catalysis, it is very likely that a web of polymorphisms among these proteins will prove to be important pharmacogenetic considerations with respect to medical diagnosis and therapy.

Some studies of the EPHX1 protein variants suggested that these coding region polymorphisms may affect protein stability. For example, in a panel of 40 human livers, EPHX1 enzyme activities demonstrated strong correlation with the respective protein levels [53]. However, neither the EPHX1 protein nor its activity was associated with the EPHX1 mRNA levels, which suggested some posttranscriptional-mechanism-regulated EPHX1 protein expression. The translational efficiency, mRNA half-life, and protein half-life of mEH allelic variants have been assessed by *in vitro* transcription and translation using constructs encoding four EPHX1 alleles. The coding region polymorphisms do not appear to affect translational efficiency or mRNA decay rate. Although the calculated EPHX1 variant protein half-lives suggested that polymorphic amino acid substitution may result in altered protein stability [145], these differences in protein half-lives were small, and it is unclear whether they affect overall functional protein levels.

As previously discussed, EPHX1 is largely responsible for the formation of PAH reactive metabolites, and fjord-region diol epoxides exhibit substantially more potent carcinogenic activities than those derived from bay regions, and fjord-region-modified DNA adducts are more difficult to repair than the bay-region adducts [79–82]. The role of the EPHX1 polymorphic variants in the formation of these carcinogenic PAH metabolites has not been assessed. It is interesting to speculate that the EPHX1 protein variants may exhibit differential catalytic activities toward these substrates, which in turn could render some individuals more susceptible to PAH-induced cancers. Similarly,

as also presented earlier, EPHX1 genotype has been implicated as a determinant of steady-state serum levels of certain anticonvulsant medications, as well as incidence of ADRs, following administration of select anticonvulsant agents. These potential functional relationships will require additional detailed study in *in vitro* assays and in clinical populations in order to ascertain.

In addition to the polymorphisms encoding amino acid change, DNA sequence variation has been identified in the 5′-flanking of human mEH, proximal to the coding region of the gene [93]. Heterologous expression of DNA fragments containing the polymorphisms indicated variable levels of reporter gene activity, suggesting that variation in this portion of the human EHPX1 gene may also contribute to functional expression levels. When considering the association of genetic polymorphisms with the risk of human disease, structural region EPHX1 polymorphisms alone likely do not account for the complete spectrum of variation influencing EPHX1 activity; polymorphisms occurring within the 5′-regulatory regions of the gene may represent additional and, perhaps, important considerations.

13.3.5 Regulation of Expression

Alterations in the EPHX1 protein level and/or activity will likely affect an individual's xenobiotic metabolism capacity. Therefore, both the genetics and regulation of EPHX1 expression in human tissues may represent an important determinant of interindividual differences in chemical responsiveness, resulting toxicities, and, perhaps, carcinogenic outcomes resulting from select chemical exposures.

13.3.5.1 Transcriptional Regulation. In rodents, EPHX1 expression can be highly inducible by a variety of compounds, including PB, 3-methylcholanthrene, polychlorinated biphenyls, *trans*-stilbene oxide [146], certain peroxisome proliferators [13], radiation [147], heavy metals [2], and select steroids [38]. In contrast, studies of human EPHX1 induction capacity have not yielded comparable results. For example, analyses of the EPXH1 transcript levels *ex vivo* in primary cultures of human hepatocytes obtained from seven individual donors subjected to *in vitro* exposures to five prototypic inducing agents, specifically, PB, dexamethasone, Aroclor 1254, ciprofibrate, β-naphthoflavone, and *bis*-hydroxyanisole, revealed that expression levels were only modestly affected by these agents in primary human hepatocytes [30]. Therefore, in this regard, the EPHX1 induction results that have been obtained in rodents cannot be easily extrapolated to humans.

13.3.5.2 Regulation by Alternative Promoters. It is well established that RNA diversity in mammals is expanded markedly through the use of alternative promoters and differential RNA splicing mechanisms, and it has been shown that these mechanisms play a role in modulating the EPHX1 gene. Initially, Gaedigk *et al*. reported complex splicing processes at the exon 1/2 boundary, which apparently generated eight putative alternative exon 1 variant sequences in addition to the well-known exon 1, E1, previously defined from liver [148]. However, it was later determined that many of the previously characterized alternative exon 1 sequences were misidentified and rather shared identity with sequences derived from the signal recognition particle 9-kDa structural gene (SRP9, NM003133), a gene that exists upstream of the EPHX1 coding region [24]. The latter study utilized a more stringent 5′-rapid amplification of cDNA

ends (RACE) technique to identify two bona fide unique first exons from human-liver-derived mRNA samples, termed *E*1 and *exon 1b* (*E*1-*b*). The E1 transcript initiated from the initially characterized promoter and was positioned immediately proximal to the exon 2 of EPHX1 coding region, while the E1-b variant exon 1 was localized to a genomic region ~18.5 kb upstream of exon 2. Northern blot RNA hybridizations demonstrated that the E1-b variant was expressed ubiquitously throughout panels of both human fetal and adult tissues. In contrast, the E1 transcript was expressed almost exclusively in liver [24]. A more recent study confirmed and extended these observations and further substantiated that the far upstream E1-b promoter functions as the primary driver of EPHX1 expression in human tissues, including liver [25]. Furthermore, genome browser sequence comparisons of the EPHX1 E1-b promoter conducted among 28 vertebrate species revealed that the E1-b promoter region was highly conserved only among primate species [94], suggesting that the regulation of EPHX1 expression in species such as mouse and rat may involve distinct promoter regulatory mechanisms. Currently, the nuclear factor interactions and associated molecular mechanisms regulating this critically important transcriptional domain of EPHX1 are under intensive investigation.

With respect to the proximal and initially characterized E1 promoter, the basis for its liver-specific functionality was examined by several investigators. A number of cis-regulatory elements were identified that appear to contribute toward its hepatic transcription character, including GATA and HNF3 motifs [24]. For example, mutation of the GATA site at position −110/−105 resulted in a marked (70%) decrease in basal transcription activities driven by the E1 promoter in human hepatoma cells, and GATA-4 was identified as the principal GATA family member interacting with the respective motif. Interestingly, both HNF3α and HNF3β were found to interact with the HNF3 element at position −96/−88, but they acted to negatively regulate GATA-4 function in hepatic cells. GATA-4 has been described previously as a regulator of EPHX1 tissue-specific expression, with assistance from other tissue-restricted transcription factors including the CCAAT/enhancer-binding protein α (C/EBPα) and nuclear factor Y (NF-Y). These factors appear to form a complex and bind directly to the CCAAT box within the E1 promoter, activating hepatic EPHX1 E1 transcription [149].

13.3.5.3 Transposable Elements. Characterization of the entire human EPXH1 gene locus has uncovered a number of remarkable features. The use of an alternative gene promoter, located ~18 kb upstream from the coding region of the gene, was described above, and it is the predominant driver of EPHX1 expression in human tissues. Another feature of the EPHX1 gene locus relates to the presence of repetitive DNA elements. Mammalian genomes are complex and dynamic, merely a small fraction is occupied by protein coding exons, while up to 50% is contributed by repetitive elements, with the remaining 48% comprising "unique DNA" [150]. Nearly half of the human genomic sequences are derived from ancient transposable elements (TEs), which encompass both transposons and retrotransposons, including short interspersed element (SINE) and long interspersed element (LINE), long terminal repeats (LTRs), and DNA transposons [151]. TEs are DNA sequences that are capable of integrating from one site in the genome to a new one via a "cut and paste" mechanism [152]. First described as "junk" DNA, the importance and function of TEs have been increasingly appreciated recently. These TEs or mobile elements participate in genome formation and benefit the evolution of genes by affecting their functions. TEs have the ability to promote

genetic diversification and regulatory variation by serving as recombination hot spots, altering protein coding content or by regulating gene transcription [153,154]. TEs have been shown to serve as alternative promoters for many genes, including aromatase P450 (CYP19; [155]), carbonic anhydrase 1 [156], and bile acid CoA: amino acid N-transferase [157], resulting in tissue-specific regulation of gene expression.

Alu elements are a particularly important type of TE and comprise the major components of SINEs that exist in the human genome; their 1.1 million copies occupy over 10% of the human genome and contribute to a significant portion of human genetic diseases [158]. It is therefore of interest and potentially of functional importance that the far upstream E1-b promoter region of human EPHX1 is replete with repetitive elements, including those of the Alu class, and that humans are genetically polymorphic for the inclusion of a specific double Alu repeat cluster [94,159]. Presence of the double repeat element appears to reduce transcriptional activity of the E1-b promoter in cell lines of different tissue lineage [94]. If these results also apply to the *in vivo* situation, differences in EPHX1 expression and corresponding capacities to conduct xenobiotic metabolism, may arise from these or other promoter region polymorphisms and may have an important bearing on interindividual risk factors associated with epoxide catalysis in target cells.

13.3.6 Summary/Pharmacological Implications

In summary, EPHX1 is an important xenobiotic enzyme, contributing critical activities toward both detoxication and bioactivation processes. The identification of human genetic polymorphisms within the EPHX1 gene locus may represent risk variables to various disease syndromes that include cancers [83], certain pulmonary diseases [132,160], preeclampsia [161], METH addiction [106], and adverse reactions to pharmacotherapies. For example, in the case of adverse drug reactions, anticonvulsant hypersensitivity syndrome has been postulated to potentially involve EPXH1 [88,89]. The transcriptional regulation of human EPXH1 appears to differ markedly from that in rodents, for example, involving the primary contribution of a far upstream gene promoter region [24,94]. Elucidating the molecular mechanisms of EPHX1 transcriptional control in human tissues and the potential genetic contribution of EPHX1 to the etiology of human disease remain important venues for future research.

13.4 SOLUBLE EPOXIDE HYDROLASE (EPHX2)

13.4.1 Introduction

Although its existence was controversial in early studies, sEH (EPHX2) was first identified as a separate enzymatic activity several years after the discovery and characterization of EPHX1. Initially, the respective enzymes were largely distinguished by their soluble fraction versus microsomal subcellular localization [55,162]. Both enzymes were originally thought to participate in xenobiotic metabolism, although EPHX2 was recognized at its inception for its role in the metabolism of a terpenoid juvenile hormone mimic in insects [55,162]. Relatively early, EPHX2 was recognized for its preference for trans- over cis-substituted epoxides of sterically hindered substrates, and hence, *trans*-stilbene oxide has often used to distinguish EPHX2 activity

from EPHX1 activity, as the latter enzyme exhibits strong preference for *cis*-stilbene oxide [54]. However, more recent experimental evidence has firmly established that the major role of EPHX2 is in the metabolism of endogenous epoxy fatty acids, with the EETs emerging as the best studied EPHX2 substrates [13]. Although the endogenous role of EPHX2's metabolism seemingly shifts it focus away from its function as a drug-metabolizing enzyme, the theme of this text, the enzyme has taken on new and important identities that position it squarely as an important target for pharmacological intervention. Therefore, these latter therapeutic dimensions of EPXH2 are the primary focus of the subsequent sections.

Human EPHX2 is localized on chromosome 8 at position 8p21-p12 [163], a locus comprising a single gene that consists of 19 exons, and encodes a 62.5-kDa protein (EC 3.3.2.3) [164]. Unlike the monomeric EPHX1 protein, the EPHX2 protein exists largely as homodimers of its monomeric subunit [165–168]. Each EPHX2 protein monomer is composed of two functional domains joined by a proline-rich linker. The C-terminal domain contains the α/β-hydrolase structure homologous to haloalkane dehalogenase and is responsible for the epoxide hydrolase activity [13,169]. The N-terminal domain is similar to haloacid dehalogenase, and functions as a lipid phosphatase [13,170,171]. EPHX2 is widely distributed in numerous tissues, with highest activity in the liver [172], followed by the kidney [173], where its distribution is concentrated within the renal cortex. The primary isolation of EPHX2 is from the cytosolic or soluble fraction, but in some cases, EPHX2 activity was reportedly localized in peroxisomes [174]. In rodents, drugs such as the peroxisome proliferator-activated receptor α (PPARα) agonists are strong inducers of EPHX2 [175,176], although functional PPARα response elements have not been identified in the upstream of human EPHX2 gene.

13.4.2 Biological Functions

Numerous investigations have now demonstrated the importance of EPHX2 in the regulation of high blood pressure [177,178] and inflammation [179,180]. Recently, with the development of EPHX2 inhibitors, the generation of EPHX2 null mice and the analysis of EPHX2 genetic polymorphisms, a more detailed understanding of the important biological role of mammalian EPHX2 has emerged.

As indicated earlier, it has become increasingly clear that fatty acid epoxides are the major endogenous substrates for EPHX2, with the cytochrome P450-derived epoxides of arachidonate acids (EETs) being the most well studied [13]. An overview of arachidonic metabolism is presented in Fig. 13.4. Numerous investigations have now demonstrated that EETs are chemical mediators that play significant roles in cardiovascular and renal regulation, modulating physiological parameters including hypertension, inflammation, angiogenesis, and mitogenic effects in the kidney [181]. Over the past several years, selective pharmacological EPHX2 inhibitors, such as 12-(3-adamantan-1-yl-ureido)-dodecanoic acid, have been designed with the potential to treat a variety of disease situations [3,114].

Several biologically imported EETs are diagramed in Fig. 13.5. Generally, the hydrolysis catalyzed by EPHX2 eliminates the biological activity of the lipids. EPHX2 was therefore postulated to play a role in blood pressure regulation, as 14,15-EET is a potent vasodilator. In subsequent studies, EPHX2 null mice were found to exhibit decreased blood pressure [177]. Moreover, recent physiological and genetic studies in a hypertensive rat model have further delineated the important role of EPHX2 in

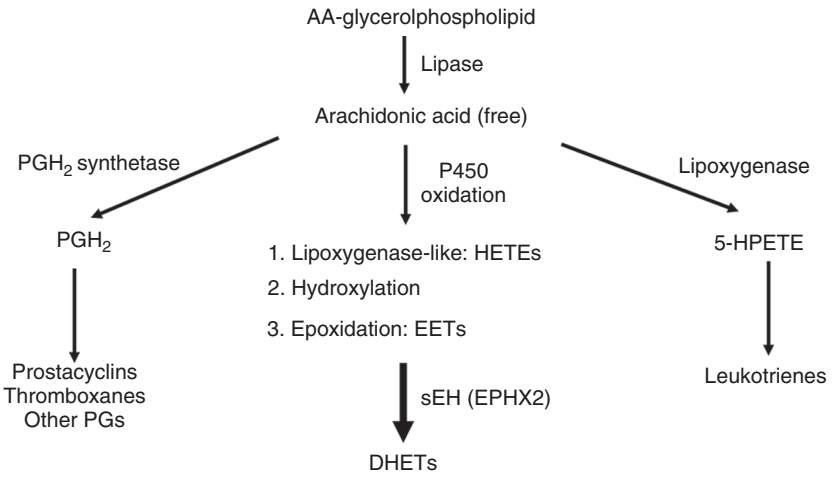

Figure 13.4 Overview of the arachidonic acid metabolism cascade. PG, prostaglandin; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid.

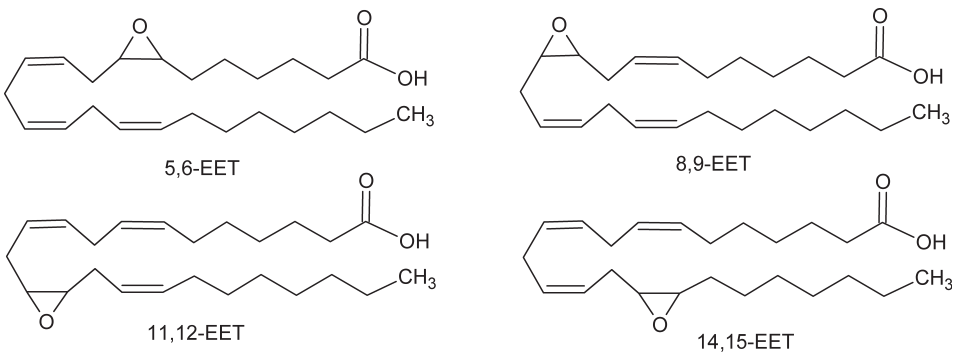

Figure 13.5 Illustration of several epoxyeicosotrienoic acid intermediates that display reactivity with EPHX2.

blood pressure regulation and as a susceptibility factor for heart failure [182,183]. Therefore, inhibition of EPHX2 is considered a new therapeutic approach for hypertension. In addition to vasodilatation, EETs also display anti-inflammatory role in vascular endothelial cells by inhibiting cytokine-induced NF-κB transcription. It has been suggested that EPHX2 may play a key role in the regulation of inflammation by metabolizing anti-inflammatory EETs and in bioactivation of the toxic and proinflammatory effects of leukotoxin. In cell culture, inhibiting EPHX2 results in EET accumulation and enhances their anti-inflammation effect [3]. Also in these respects, investigations conducted in null mouse models have demonstrated that EPHX2 deletion attenuates renal injury and inflammation in a high salt model [184].

Similarly, on the basis of studies conducted in cardiovascular models of ischemic injury and hypertrophy, EPHX2 has emerged as a potentially important pharmacological target in cardioprotection. Indications are that inhibitors of enzyme may offer therapeutic potential for a broad range of cardiovascular diseases [185]. As endothelial dysfunction contributes to the development of coronary heart disease, contribution

of EPHX2 in the metabolism of the EETs in vasculature tissues is likely an important factor in the regulation of endothelial function [186]. Interestingly, EPHX2 was recently identified as a heart failure susceptibility gene. These studies reported that the metabolism of cardioprotective EETs are affected by EPHX2 allelic variants that result in altered transcript and protein levels [183].

13.4.3 Genetic Polymorphism

Approximately 755 single nucleotide polymorphisms (SNPs) for human EPXH2 are cataloged currently in the NCBI SNP database. However, the great majority of these SNPs reside in noncoding regions of the gene or code for synonomous nucleotide substitutions. Initial analyses of EPHX2 genetic polymorphism in humans revealed the existence of two nonsynonomous polymorphisms, resulting in altered human sEH/EPHX2 amino acid sequences with associated alteration in enzymatic function or stability [187]. One involved a polymorphism at residue 287 (Arg287Gln; rs751141), where the more common arginine is nonconservatively substituted with glutamine [187,188]. A second coding region variant (rs71868122) involved an unusual trinucleotide insertion encoding an additional arginine residue, following the normal serine in position 402 [187]. Additional nonsynonomous polymorphisms have since been identified, including a Lys55Arg (rs4150795) amino acid substitution. All these structural EPHX2 polymorphisms appear to affect enzymatic function. Results of *in vitro* enzymatic assays as well as comparative molecular modeling analysis based on the crystal structure of EPHX2 indicate that the Arg287Gln variant possesses decreased activity as well as decreased protein stability relative to the reference protein [187,189]. On the other hand, the Lys55Arg variant appears to encode a more highly active form of EPHX2 [186,189].

Results of molecular epidemiological studies in human populations assessing the potential association of EPXH2 genetic polymorphism in human disease have yielded interesting findings. For example, in a study of risk factors associated with familial hypercholesterolemia, plasma cholesterol levels were elevated in individuals carrying both the Arg287 allele and a mutation in the low density lipoprotein receptor (LDLR) [190]. Interactive effects of Arg287 with carriers of the LDLR polymorphism were also noted between increased risk of cardiovascular disease and incidence of familial hypercholesterolemia [190]. The R287Q polymorphism was further reported as associated with carotid artery calcified plaque disease in European Americans [191]. Furthermore, the Lys55Arg polymorphism has been reported as a risk factor in coronary heart disease [186]. In white Americans, Lys55Arg genotype was associated with vasodilator response to bradykinin, such that forearm blood flow was significantly lower ($P = 0.043$) and forearm vascular resistance was significantly higher ($P = 0.013$) in Arg55 variant allele carriers compared to wild-type individuals. Significant associations were also observed with methacholine and sodium nitroprusside, suggesting an important role for sEH in the regulation of vascular function in humans [192]. As these are complex disease phenotypes, likely dependent on the regulation of eicosanoid metabolism that involves both cytochrome P450 epoxidation to generate the EETs as well as EPHX2 hydrolysis in the degradation of EETs, the exact genetic relationships that dictate interindividual risk differences in these disease etiologies will require an additional detailed study.

13.4.4 Regulation of Expression

Although the biological function of human EPHX2 has been extensively investigated, much less is known about the molecular mechanisms of EPHX2 regulation. Several observations in rodent models have shown that EPHX2 can be regulated by endogenous chemical mediators and some xenobiotics. For example, both cigarette smoke and γ radiation appear to affect human EPHX2 expression levels [193,194]. It has also been noted that male mice exhibit higher EPHX2 activities in liver and kidney than female mice [13]. These observations suggest that sex hormones may be involved in EPHX2 regulation, at least in mice.

In rodents, EPHX2 expression is induced by the administration of PPARα agonists. Peroxisome proliferators are compounds that induce the size and number of hepatic peroxisomes. PPARα agonists include clinically used hypolipidemic drugs, endogenous compounds such as steroids, dietary fatty acids, and commercial plasticizers [195]. The response to these agonists suggests that EPHX2 may play role in peroxisome proliferator-induced liver toxicity in rodents. However, whether these responses are maintained in humans is an important consideration and one that has not been definitively evaluated. To this effect, the core promoter of the human EPHX2 gene was characterized recently by the Hammock group [196]. No PPARα responsive motifs were identifiable in the human gene's promoter region. However, nuclear factor Sp1 regulatory elements were apparent that were localized in a GC-rich region of the promoter and appear to be important contributors to the basal levels of EPHX2 transcriptional regulation.

Another recent report indicated that angiotensin II (Ang II), a potent vessel constrictor that elevates blood pressure in animal models, directly upregulates EPHX2 transcription expression mediated by an AP-1 motif present in the human EPHX2 promoter [197]. These investigators further demonstrated that the transcriptional regulation mediating EPHX2 induction by Ang II involved c-Jun/c-Fos binding to the AP-1 site at the -446 position of the gene's promoter [197]. In addition, overexpression of a mutant c-Jun protein that was lacking its respective transactivation domain markedly attenuated the EPHX2 induction response otherwise facilitated by Ang II.

Alternative splicing of RNA transcripts may also play a role in the regulation of EPHX2 expression. Interestingly, in mouse ovary, a unique variant of EPHX2 was reported as generated by alternative splicing. This variant lacked the associated phosphatase activity normally encoded in the transcript [198]. This short isoform of EPHX2 possesses an altered N-terminal sequence spliced from the second intron. Examination of the alternative splicing databases further predicts a number of potential EPHX2 splice variants in humans; however, most of these variants would be predicted not to be functional as a consequence of the deletion of entire exons or due to the introduction of stop or out-of-frame codons. To our knowledge, no publications on splice variation in human EPHX2 have been reported. This area remains to be one requiring more detailed study.

13.4.5 Summary/Pharmacological Implications

Although originally thought of as a xenobiotic-metabolizing enzyme, this is no longer considered a functional role for EPHX2. Rather, this sEH has been identified as a major contributor to the metabolism of endogenous substances, in particular, epoxides derived

from the arachidonic acid cascade. In these respects, EPHX2 appears to contribute important biological functionality in processes that include control of blood pressure and inflammatory responses. Thus, the enzyme has been targeted by pharmacological modulators, facilitated by the development of highly selective and potent inhibitors of the enzyme that are currently in clinical trials. Historically, EPHX2 represents one of the few cases where an enzyme originally described as being involved in xenobiotic metabolism has evolved as an exciting clinical target.

13.5 CONCLUSIONS

Together, the epoxide hydrolases, in particular, the microsomal (EPXH1) and soluble (EPXH2) forms of the enzyme, have achieved prominent recognition for their respective biological roles in xenobiotic and endogenous metabolism. As outlined in this chapter, these enzymes also contribute to the spectra of drug metabolism, with EPXH1 involved in the metabolism of several drug substrates and EPXH2 as a pharmacological intervention target and mediator of physiological processes such as control of blood pressure and inflammation. Although the history of discovery and characterization of these respective enzyme systems is rich, a great deal of additional study is required to fully elucidate their extended functional roles, the details of their transcriptional control, and the influence of genetic polymorphism present in these systems as potential risk modifiers in pharmacotherapy and chemically induced disease.

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