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Soluble epoxide hydrolase: Gene structure, expression and deletion

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Abstract

Mammalian soluble epoxide hydrolase (sEH) converts epoxides to their corresponding diols through the addition of a water molecule. sEH readily hydrolyzes lipid signaling molecules, including the epoxyeicosatrienoic acids (EETs), epoxidized lipids produced from arachidonic acid by the action of cytochrome p450s. Through its metabolism of the EETs and other lipid mediators, sEH contributes to the regulation of vascular tone, nociception, angiogenesis and the inflammatory response. Because of its central physiological role in disease states such as cardiac hypertrophy, diabetes, hypertension, and pain sEH is being investigated as a therapeutic target. This review begins with a brief introduction to sEH protein structure and function. sEH evolution and gene structure are then discussed before human small nucleotide polymorphisms and mammalian gene expression are described in the context of several disease models. The review ends with an overview of studies that have employed the sEH knockout mouse model.

Keywords

EPHX2; epoxyeicosatrienoic acid; lipid signaling; inflammation; hypertension

1. Introduction

This review first summarizes the role of sEH in the hydrolysis of epoxy fatty acids (EpFA), the proposed endogenous substrates of the enzyme. We then provide a comprehensive evaluation of sEH gene expression and regulation, including its localization in mammalian tissues. Finally, we discuss sEH genetic models that have associated sEH with diseases such as cardiovascular disease, cancer and diabetes as well as pain, and have contributed to the identification of the enzyme as a pharmaceutical target.

2. sEH protein structure and catalytic activities

Human sEH is a 62kDa enzyme composed of two domains separated by a short proline-rich linker (see Figure 1) (Newman et al., 2005). In mammals, sEH is a homodimer in the intracellular environment (Morisseau and Hammock, 2012). The N-terminal domain exhibits a phosphatase activity that hydrolyzes lipid phosphates, while the C-terminal domain

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exhibits an epoxide hydrolase activity that converts epoxides to their corresponding diols (see Figure 2) (Morisseau and Hammock, 2012). sEH hydrolyzes EpFA, including one important class of lipid signaling molecules, the epoxyeicosatrienoic acids (EETs) (Spector and Norris, 2007). EETs have vasoactive, anti-inflammatory and analgesic properties (Imig, 2012). Through metabolism of these molecules and other EpFA, sEH is implicated in several diseases, including hypertension, cardiac hypertrophy, arteriosclerosis, brain and heart ischemia/reperfusion injury, cancer and pain (Imig and Hammock, 2009; Wagner et al., 2011; Morisseau and Hammock, 2012). Because of its possible role in cardiovascular and other diseases, sEH is being pursued as a pharmacological target, and several potent small molecule inhibitors are available (Shen and Hammock, 2012).

3. Gene evolution

The two domain, bi-functional mammalian sEH is the product of a gene fusion event (see Figure 3) (Beetham et al., 1995). The C-terminal domain exhibits homology with bacterial haloalkane dehalogenase, as well as single domain epoxide hydrolase enzymes in fungi, plants, and invertebrates such as *Caenorhabditis elegans* (*C. elegans*) (Newman et al., 2005; Harris et al., 2008b). The N-terminal domain of the mammalian enzyme is homologous to the bacterial haloacid dehalogenase (Beetham et al., 1995). Homologs of the phosphatase domain exist in the genome of archaea, bacteria and *C. elegans* (Cronin et al., 2003; Harris et al., 2008b).

Potential selective advantages of the fused gene include the proximity of phosphatase and epoxide hydrolase activities in the bi-functional enzyme that could allow shuttling of an unknown substrate (Argiriadi et al., 1999). However, the early fused homologs characterized in chicken and sea urchin do not possess phosphatase activity (see Figure 2) (Harris et al., 2008a; Harris et al., 2008b). Interestingly, the sea urchin contains two two-domain homologs of sEH, and neither possesses N-terminal phosphatase activity, indicating that the phosphatase activity is the result of evolutionarily recent mutations in the gene or was lost and subsequently regained (Harris et al., 2008a). Other possible selective advantages of gene fusion include the promotion of dimerization and enzyme stability (Beetham et al., 1995; Argiriadi et al., 1999).

4. Mammalian gene structure

The mouse gene is located on chromosome 14 and consists of 19 exons that encode for 554 amino acid residues (Grant et al., 1993; Grant et al., 1994). The transcript was first cloned from mouse liver (Grant et al., 1993; Grant et al., 1994). A reported mouse splice variant contains a sequence located in the second intron of the gene in place of the first two exons (Hennebold et al., 2005). This splice variant lacks residues important for phosphatase activity, and is expressed in granulosa cells of preovulatory follicles.

The human gene is composed of 19 exons located on chromosome 8 that encode for 555 amino acid residues (Beetham et al., 1993; Larsson et al., 1995; Sandberg and Meijer, 1996). The human promoter lacks a TATA box and contains several SP-1 sites (Tanaka et al., 2008). One SP1 site immediately upstream of the transcription start site is required for minimum promoter expression. Methylation of these SP-1 sites is involved in sEH gene silencing in HEPG2 cells (Zhang et al., 2010). AP-1 sites are implicated in the upregulation of sEH by angiotensin-II (Ai et al., 2007). The sEH human promoter contains unfolded protein response elements (UPRE) that upregulate sEH in human umbilical vein endothelial cells (HUVECs) in response to homocysteine (Hcy) treatment (Zhang et al., 2012a). Hcy significantly upregulates sEH mRNA expression, partially through the action of the activating transcription factor-6 (ATF-6). The binding of ATF-6 is enhanced by Hcy-mediated demethylation of the binding site.

There are a number of physiologically relevant single nucleotide polymorphisms (SNPs) in rat and human populations (Sandberg et al., 2000; Saito et al., 2001; Fornage et al., 2002; Przybyla-Zawislak et al., 2003). These SNPs fall within introns and exons. Two of these mutants impact sEH catalytic activities. R287Q, within the C-terminal domain of the enzyme, has decreased epoxide hydrolase and phosphatase activity, while K55R, within the N-terminal of the enzyme, has reduced phosphatase activity when compared to the most frequent allele (Przybyla-Zawislak et al., 2003; Srivastava et al., 2004).

5. Physiological role of sEH in mammals

sEH, through its epoxide hydrolase activity, metabolizes EpFA such as the EETs. EETs are autocrine and paracrine lipid signaling molecules produced from arachidonic acid (ARA) through the action of cytochrome P450s (CYPs) that epoxidize the double bonds to form four regioisomers (see Figure 3) (Spector and Norris, 2007; Imig, 2012). ARA is also the precursor of the prostaglandins and the leukotrienes. These lipids play a role in asthma, pain, and inflammation (Haeggstrom and Funk, 2011; Smith et al., 2011). The EET receptor or receptors are unknown, but the use of sEH inhibitors, EET mimics and the EETs themselves has demonstrated the anti-inflammatory and vasoactive properties of the EETs in *in vitro* models such as isolated coronary rings and platelet aggregation assays, as well as *in vivo* models such as angiotensin-II and dietary salt-induced hypertension (Spector and Norris, 2007).

A simple model of sEH function involves the regulation of blood pressure in the kidney (see Figure 4). By hydrolyzing the vasodilatory EETs, sEH produces dihydroxyeicosatrienoic acids (DHETs), diol species that are both less lipophilic and more readily conjugated and so more easily removed from the site of action and excreted than the EETs (Morisseau and Hammock, 2012). In a simplified model, by removing this lipid mediator, sEH changes the balance of vasoconstrictive and vasodilatory signals, thereby influencing vascular resistance to blood flow and blood pressure (Morisseau and Hammock, 2012). Reduction of sEH epoxide hydrolase activity, either through a small molecular inhibitor, or through genetic knockout can stabilize or increase levels of EETs and other EpFA and so reduce hypertension (Morisseau and Hammock, 2012). The sEH inhibitors appear most effective in reducing angiotensin driven hypertension (Morisseau and Hammock, 2012). Increasing endogenous EpFA chemical mediators by decreasing their rate of hydrolysis is the molecular basis of the therapeutic action of sEH inhibitors in many disease models.

The situation *in vivo* is more complicated. EETs have displayed different properties in different vascular beds, and the products of the hydrolysis, the DHETs, are poorly characterized (Morisseau and Hammock, 2012). sEH hydrolyzes other lipid epoxides such as the ω-3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) epoxides, and the 18-carbon leukotoxin and isoleukotoxin (see Figure 2) (Zheng et al., 2001). The 18:2 diol or the bisepoxide can be further metabolized to tetrahydrofuran diols that display reproductive and behavioral properties *in vivo* (Moghaddam et al., 1996; Markaverich et al., 2007). In addition, sEH hydrolyzes all other EpFA tested at varying rates (Morisseau et al., 2010). In the linoleate epoxide series the resulting diol is thought to be a potent chemical mediator needed for normal development, acting to increase vascular permeability in some tissues (Fromel et al., 2012), but when at high concentrations it is a marker of acute respiratory distress syndrome (ARDS), promoting perivascular and pulmonary edema (Moghaddam et al., 1997). The regioisomers of the ω-3 epoxides DHA and EPA are more rapidly hydrolyzed than the corresponding ω-6 ARA epoxides, except for the slowly hydrolyzed epoxide in the ω-3 position (Morisseau et al., 2010). These ω-3 epoxides of DHA and EPA and possibly the poorly studied corresponding diols can be more potent than the EETs in some *in vitro* models (Spector, 2009). EPA epoxides inhibit endothelial cell proliferation

(Cui et al., 2011). In a number of *in vivo* rodent models the DHA epoxides seem to have similar biological effects as the EETs, however they appear to be more potent. These include reduction in enhanced pain perception or nociception (Morisseau et al., 2010) and reduction in angiotensin-II driven blood pressure (Ulu et al., 2013). Interestingly, with regard to angiogenesis, the EETs are mildly pro-angiogenic, resulting in enhanced wound repair but accelerated growth of solid tumors (Panigrahy et al., 2012). In contrast, the DHA epoxides are dramatically anti-angiogenic, acting in a non-VEGF mediated pathway. Stabilization of the ω-3 DHA epoxides results in a blockage of tumor growth and metastasis in murine xenographs or breast and lung tumors (Zhang et al., 2013.).

6. Single Nucleotide Polymorphisms

sEH SNPs were first associated with coronary heart disease in the Coronary Artery Risk Development in Young Adults (CARDIA) and the Atherosclerosis Risk in Communities (ARIC) studies (Fornage et al., 2004; Lee et al., 2006). In the CARDIA study, the R287Q allele was associated with coronary artery calcification (CAC) in African Americans, but not Caucasians (Fornage et al., 2004). The risk for CAC associated with sEH SNPs was highest among smokers (Wei et al., 2007). R287Q was not associated with hypertension in a smaller African American population (Dreisbach et al., 2005) Similarly, R287Q was not associated with ischemic stroke, myocardial infarction, and ischemic heart disease in a Danish population (Lee et al., 2010). The K55R allele is associated with the risk of developing coronary heart disease in Caucasians, but not African Americans (Lee et al., 2006). A group examining the R287Q and K55R SNPs recapitulated these results in a Swedish population (Fava et al., 2010). K55R was associated with a higher risk of hypertension and ischemic stroke in male homozygotes. When forearm blood flow and vascular resistance in black and white Americans was measured, the K55R mutant was associated in white Americans with lower blood flow and high vascular resistance in response to bradykinin, but not in black Americans (Lee et al., 2011). R287Q was associated with lower vascular resistance in response to bradykinin in black, but not white Americans.

Variations in the sEH gene are associated with other disease states, including ischemic stroke and diabetes. Two haplotypes in African American populations are associated with either increased or decreased risk of ischemic stroke (Fornage et al., 2005). Three SNPs in the sEH gene are associated with ischemic stroke. Two SNPs were located in introns, but the third was R287Q (Gschwendtner et al., 2008). Neuronal cells transfected with the R287Q mutant exhibit increased survival after oxygen-glucose deprivation, supporting a possible role for sEH in neuron survival (Koerner et al., 2007). The R287Q SNP is associated with a lower risk of ischemic stroke in a Chinese population, but only in the case of nonsmokers (Zhang et al., 2008a). R287Q was associated with insulin resistance in patients with type 2 diabetes (Ohtoshi et al., 2005). Finally, no association was indentified between K55R and hypertension or coronary heart disease in patients that received restenosis after percutaneous coronary intervention (PCI, also called coronary angioplasty) (Kullmann et al., 2009).

Interestingly SNPs in the non-coding regions of the sEH gene, as well as in the 5’ and 3’ UTR, are associated with human diseases. SNPs located in introns are associated with carotid and coronary artery calcified plaques, as well as carotid artery intima-media thickness in African and European Americans participating in the Diabetic Heart Study (Burdon et al., 2008). A SNP in the 3’ region of the gene is associated with decreased semen quality and male infertility in a Chinese population (Qin et al., 2012). In rodents, variation in the sEH promoter region segregates with heart failure in the spontaneous hypertensive heart failure rat model (Monti et al., 2008). This variation results in increased gene expression and epoxide hydrolase activity. The authors further show that sEH gene expression in humans with heart failure is downregulated relative to control patients. Finally, a mutation in the 3’

UTR is associated with differences in kidney function in patients that have undergone kidney transplant. Specifically, the absence of this SNP is predictive of kidney failure (Lee et al., 2008). These “non-coding” SNPs may play a role in sEH regulation through microRNA or other mechanisms.

Taken together, these data show that the race and sex of patients, as well as patient history must be considered when assessing the effect of sequence variation in the sEH gene. Coronary heart disease is associated with R287Q, a SNP that displays decreased sEH epoxide hydrolase activity. In addition, K55R, a SNP that displays decreased phosphatase activity, is associated with ischemic stroke. Many of these studies need to be repeated with higher numbers of patients and more refined criteria of disease. There is a critical need to follow up on these observations with experimental models in animals and clinical interventions in man.

7. Mammalian gene expression

Introduction

sEH was first identified through its activity on substrates such as juvenile hormone, as well as lipid epoxides such as epoxystearate (Hammock et al., 1976). Through gel filtration and the assessment of differential hydrolysis of epoxystearate, the epoxide hydrolase activity in soluble fractions was determined to be distinct from the microsomal fraction obtained from the liver and kidneys of mice (Gill and Hammock, 1979). Subsequent studies using a lipid epoxide as a substrate detected this activity in the soluble fraction of multiple organs, though at a lower level than in liver and kidney (Gill and Hammock, 1980). The enzyme activity was detected in rabbits, mice and rats, and the proposed enzyme was named soluble epoxide hydratase or hydrase. Establishment of systematic nomenclature by the International Union of Pure and Applied Chemistry has lead to the acceptance of soluble epoxide hydrolase as the name of the enzyme.

There are sEH homologs in plants such as potato and cress, and animals such as nematode, sea urchin, chicken, cat, and dog where it was cloned and expressed (Morisseau and Hammock, 2012). Although sEH activity is highest in the liver and kidney, it is implicated in important physiological processes in the brain and lung and other organs. sEH was first identified and localized to tissues through its epoxide hydrolase activity. To focus this review, we have confined our discussion to sEH mRNA and protein expression. A summary of the significant results discussed in this section, as well as additional results not discussed, are contained in Table 1.

Subcellular localization

Originally identified in the soluble fraction of organ lysates, the difficulties of subcellular fractionation and/or the permeability of subcellular compartments lead to contradictory results. After the initial reports of sEH activity, sEH was detected in mitochondrial, microsomal and cytosolic fractions by activity or in the peroxisomes alone by immunohistochemistry (Waechter et al., 1983; Kaur and Gill, 1986; Hollinshead and Meijer, 1988). Eventually it was determined that the activity in the mitochondrial fractions was an artifact from peroxisomal contamination, and the same enzyme was responsible for the activity in the cytosol and peroxisomes (Chang and Gill, 1991; Eriksson et al., 1991). The small amount of sEH either trapped in the microsomes or tightly associated with them can be differentiated with selective substrates or inhibitors. A more recent comprehensive examination of multiple tissues reported that sEH peroxisomal localization may be tissue specific (Enayetallah et al., 2006a). The peroxisomal localization is complex because targeting sequences in the mammalian sEH are perfect glyoxysome targeting signals in plants but imperfect in mammals.

sEH, PPAR agonists, and Angiotensin-II

A challenge facing the investigation of sEH in isolated cells is that sEH is not uniformly expressed in immortalized cell lines. In addition, most cell lines do not express a full complement of CYPs, major producers of the EETs and other EpFA that are substrates for sEH (Langenbach et al., 1992; Tanaka et al., 2008). However, in the absence of good cell-based systems, a number of inducers were identified *in vivo*. Most recently, treatment with Arsenic-III increased sEH mRNA expression in the lung, liver and kidney of mice (Anwar-Mohamed et al., 2013). The protein levels increased in the lung, but they did not change in the liver and decreased in the kidney.

The first identified inducer of sEH activity was clofibrate, a peroxisome proliferator that increased sEH activity in rodent liver (Hammock and Ota, 1983). Clofibrate induces sEH mRNA expression in intestine, kidney and heart, as well as the liver of mice (Johansson et al., 1995). Interestingly, the human 5' UTR does not contain PPARα response elements (Tanaka et al., 2008).

PPARγ activation is linked to sEH expression in human endothelial cells (Liu et al., 2005). The laminar flow produced by a flow chamber apparatus simulates anti-inflammatory pathways in these cells and activates PPARγ. In this model, sEH mRNA and protein expression is downregulated under conditions of increased laminar flow and the inhibition of sEH epoxide hydrolase activity further enhances the PPARγ response. A mouse heterozygote for a PPARγ deletion has reduced sEH mRNA expression in the heart (Pang et al., 2011). Additionally, in an angiotensin-II infused rat model, the increase in sEH protein levels can be reversed by treatment with the PPARγ agonist rosiglitazone (Pang et al., 2011). Rosiglitazone increases mouse sEH mRNA expression in epididymal and subcutaneous fat pads, but not in the liver and kidney (De Taeye et al., 2010). The link between PPARγ activation and sEH expression is an ongoing area of research (Imig et al., 2012; Nayeem et al., 2012; Xu et al., 2012).

Angiotensin-II (Ang-II) is a peptide hormone with vasoconstrictive properties involved in the regulation of blood pressure and the pathology of hypertension. Ang-II treatment of HUVECs results in an over two-fold increase in mRNA expression of sEH (Ai et al., 2007). The authors attribute this increase to the action of c-Jun binding to putative AP-1 binding sites located in the human sEH promoter region, since deletion constructs of the promoter lacking AP-1 sites lose their response to Ang-II. ChIP assays confirmed that cJun binds to two elements in the promoter. This result was replicated in rat endothelial cells, and in two *in vivo* models, where sEH transcript expression is specifically upregulated in the rat aortic intima.

Receptor-dependent regulation of sEH was apparent since the enzyme was first identified in rodents. In human, exploration of the role of PPAR receptors in the regulation of sEH expression will provide a connection between EpFA signaling and lipid homeostasis and pathologies such as metabolic syndrome. The involvement of sEH in the renin-angiotensin system (RAAS) has wide ranging implications for inflammatory diseases and hypertension.

Vasculature

sEH is implicated in the hydrolysis of the vasoactive EETs. sEH SNPs are associated with cardiovascular disease. Taken together, these data suggest a role for sEH in the vasculature. sEH is localized in the rat mesenteric artery (Zhao et al., 2005; Olearczyk et al., 2006). Vasodilatory effects of sEH inhibitors are not affected when the endothelium is stripped from isolated rat vessels, indicating that sEH in the vascular smooth muscle may be responsible for the vasodilatory effects (Olearczyk et al., 2006). (An aside for future *in vitro* experiments: at least some cultured VSMCs do not contain sEH or lose the enzyme after

repeated passages (Davis et al., 2006).) In the obese Zucker rat, the mesenteric microvessel endothelium-dependent dilation is reduced relative to lean Zucker rats (Zhao et al., 2005). Interestingly, sEH is upregulated in the mesenteric arteries of these rats (Zhao et al., 2005). Treatment with ethinyl estradiol, tamoxifen, and raloxifene in ovariectomized rats upregulates sEH mRNA expression in mesenteric arteries (Mark-Kappeler et al., 2011). sEH protein is expressed in red blood cells (Jiang et al., 2008). sEH mRNA and protein expression is decreased in the red blood cells (RBCs) in the spontaneous hypertensive rat, compared to the Wistar control (Jiang et al., 2011).

These results support the involvement of sEH in paracrine and autocrine signaling in the vasculature, through its metabolism of vasoactive EpFA signals produced in the region of VSMCs and endothelial cells. The pool of sEH in RBCs could exert its effects systemically, having enhanced effects when blood cells are in close proximity to vessel walls, such as occurs in the micro-capillaries of peripheral tissues.

Heart

sEH is expressed in the aorta and in both atrial and ventricular tissues of rodents (Xu et al., 2006; Chawengsub et al., 2008). Because the EETs display vasoactive properties on isolated coronary artery rings, the expression of sEH in coronary arteries is of special interest (Fang et al., 1999). In endothelial nitric oxide synthase (eNOS) knockout animals fed a high salt (HS) diet, sEH protein expression in the aortas is reduced in HS-fed eNOS-/- and eNOS+/+ mice relative to mice fed a normal salt diet, with the most significant reduction in the eNOS +/+ mice (Nayeem et al., 2011). This link between eNOS and sEH expression implies that there is interplay between different endothelium-derived hyperpolarization factors in models that exhibit endothelium dysfunction. For example, exposure to tobacco smoke upregulates sEH mRNA expression in the endothelial cells of aortas of mice (Maresh et al., 2005) and hypertension induced by renal artery stenosis increases sEH protein expression in the endothelial cells of coronary arteries of mice (Gao et al., 2011). In summary, sEH is implicated in proper functioning of the endothelium of coronary arteries and the aorta.

The SNPs identified in the CARDIA and ARIC studies suggest that variation in sEH epoxide hydrolase activity may play a role in cardiovascular disease. sEH expression is modulated in the heart in several disease models, prominently in models of cardiac hypertrophy.

sEH protein levels increase in the heart of a rat Ang-II induced model of cardiac hypertrophy (Ai et al., 2009). This increase in sEH was not detected in hypertrophy induced by norepinephrine or in so-called physiological hypertrophy induced by exercise training. Increased expression of sEH mRNA and protein also occurs in the heart in a rat isoproterenol-induced model of cardiac hypertrophy (Zordoky et al., 2008; Althurwi et al., 2012). There are conflicting reports of sEH expression in mouse pressure-induced models of hypertrophy, where no change or a reduction in sEH protein and mRNA expression is detected in heart tissue (Xu et al., 2006; Morgan et al., 2012). Chronic treatment with doxorubicin increases hypertrophic, inflammatory and apoptotic markers as well as sEH mRNA expression in the rat heart (Alsaad et al., 2012). Finally, Arsenic-III treatment increases mRNA expression of hypertrophic markers as well as protein and mRNA levels of sEH in the mouse heart (Anwar-Mohamed et al., 2012). These data imply a distinct role for sEH in the progression of pathological hypertrophy.

sEH is not upregulated in norepinephrine-induced or pressure-induced hypertrophy or so-called physiological hypertrophy, indicating that sEH is not merely a marker of hypertrophy, or even a general marker of pathological hypertrophy, as is the fetal gene marker beta myosin heavy chain, for example. Its upregulation in the heart in Ang-II induced

hypertrophy shows a further link between the renin-angiotensin-aldosterone system (RAAS) and sEH, and demonstrates that sEH is involved in specific physiological effects of chronic disregulation of the RAAS. EpFAs appear so intimately involved in the RAAS that possibly it should be termed the ERAAS to indicate the importance of EpFA in the system.

Use of sEH inhibitors and sEH genetic models has implicated the enzyme in cardiac ischemia/reperfusion injury. However, sEH protein levels are not upregulated in the mouse left ventricle free wall in a myocardial infarction model (Li et al., 2009). Similarly, sEH mRNA expression does not change in the left ventricle in a rat chronic heart failure model (Merabet et al., 2012).

Kidney

sEH is expressed in the renal vasculature as well as the tubules to a lesser degree, as determined by immunohistochemistry on human kidneys (Yu et al., 2004). The weaker immunoreactivity in the glomeruli and tubules is homogeneous, but in the arteries sEH is localized to the smooth muscle layers of the arterial wall (Yu et al., 2004). In mice, sEH mRNA and protein expression is greater in the kidneys of male mice, compared to female (Sinal et al., 2000; Chanas et al., 2003).

Female and male spontaneously hypertensive rats (SHR) have elevated levels of sEH mRNA and protein in the kidney cortex compared to Wistar-Kyoto “control” rats (WKY) (Yu et al., 2000; Koeners et al., 2011). In a cDNA microarray experiment, sEH mRNA expression was increased in the SHR kidney compared to the WKY controls and sEH was the primary outlier at 3 and 9 weeks of age (Seubert et al., 2005). sEH protein expression is greater in male SHR kidneys compared to females, and ovariectomy increases the expression (Martin et al., 2012). This difference in the sexes mirrors the situation in the mouse.

Another model of hypertension maintains Ang-II infusion via an implanted osmotic mini-pump. In general, Ang-II infusion in rats increases sEH protein expression in the renal cortex and microvasculature (Imig et al., 2002; Zhao et al., 2004). In Ang-II infused mice, sEH is highly expressed in the distal tubuli and expressed at lower levels in the glomeruli, as determined by immunohistochemistry (Jung et al., 2005). Similarly, in the two-kidney-one-clip model of hypertension, sEH is upregulated in the renal cortex (Walkowska et al., 2010; Kopkan et al., 2012). These results point to a role for sEH in the vasculature of the kidney in the maintenance of hypertension. Interestingly, there is no difference in sEH protein expression in the kidney cortex and medulla of transgenic mice with inducible expression of the renin gene when the control and induced groups are compared (Honetschlagerova et al., 2011). Similarly, sEH expression in the kidney did not change in a dietary salt-loading model of hypertension (Sinal et al., 2000). The specific localization and high expression of sEH in the kidney is an indication of the important role sEH plays in the control of blood pressure, presumable through its ability to metabolize EpFA with osmoregulatory and vasoactive properties.

In rodent models of diabetes, sEH expression varies from no change to a decrease in expression in the kidney. Fructose-fed insulin resistant hypertensive rats do not exhibit a change in sEH protein expression in whole kidney preparations (Mustafa et al., 2010). However, sEH protein and mRNA expression is decreased in the kidneys of diabetic mice in a streptozotocin (STZ)-induced mouse model, where sEH is immunolocalized prominently to the proximal tubules (Oguro et al., 2009). This illustrates the difficulty in comparing immunohistochemistry to data from tissue homogenates in highly structured organs such as the kidney. Because of the localization of sEH, whole tissue homogenates may mask important changes in expression. Relevant to these diabetic models, sEH is expressed in

human pancreatic beta islet cells (Enayetallah et al., 2004). Finally, modulation in sEH expression is linked to kidney damage. First, there is a decrease in renal levels of sEH mRNA and protein in a mouse model of progressive renal disease where one kidney is surgically impaired and damaged and the second kidney is removed (Jung et al., 2010). A second group has recently reported an increase in sEH protein expression in the renal proximal tubules of human patients with glomerulonephritis (Wang et al., 2012a). They also report an increase in sEH protein expression in the renal cortex of mice with adriamycin-induced nephritis. These results raise the general issue of EpFA and organ protection. It is possible that EpFA play a role in the protection of particular organs from damage due to chronic disease states as well as acute injuries.

Liver

Epoxide hydrolase activity is present in the cytosolic fractions prepared from the livers of monkeys and rodents. This activity is upregulated by clofibrate and disease states and conditions such as STZ-induced diabetes and food restriction (Hammock and Ota, 1983; Pacifici et al., 1983; Moody et al., 1985; Thomas et al., 1989). Treatment with xenobiotics such as the drug doxorubicin or the environmental toxin acrylonitrile does not alter sEH expression in the rat liver (Chanas et al., 2003; Zordoky et al., 2011). Mirroring the results from kidney, there is a difference in sEH expression in males and females; male rats have greater sEH protein expression than female rats in the liver (Chanas et al., 2003).

sEH expression levels have both increased and decreased in models that involve modulation of dietary fat. In a mouse high-fat (HF)-diet-induced fatty liver model, a 16 week diet increased the expression of sEH mRNA in the liver (Liu et al., 2012). This model used a 60% versus 10% fat diet of unspecified composition. When the fatty acid composition but not the total amount of fat in the diet is manipulated, supplementation with fish oil decreases the mRNA and protein levels of sEH in the mouse liver compared to animals fed a diet supplemented with a high-oleic acid sunflower-seed oil or DHA-rich oil (Mavrommatis et al., 2010). The authors conclude that EPA may be responsible for the decrease in expression of hepatic sEH. This result indicates that researchers who use high fat diets in their disease models should carefully examine the composition of the added fat, since it appears that sEH expression in the liver may be altered by specific lipids. This is not surprising, since several different classes of fatty acids can be metabolized by CYPs to form potential EpFA signaling molecules and some of these are substrates for sEH, such as the epoxides of the omega three fatty acids DHA and EPA, the epoxides of linoleic acid, as well as the EETs (Morisseau et al., 2010) (see Figure 2).

Brain

In the human brain, sEH is immunolocalized primarily in astrocytes, oligodendrocytes and neuronal cell bodies (Sura et al., 2008). sEH is also detected in the smooth muscle cells of arterioles in the brain vasculature (Sura et al., 2008). In rat cerebral parenchymal microvessels, sEH is immunolocalized to the vascular smooth muscle cells (VSMCs) of the microvasculature and the perivascular nerves that innervate the middle cerebral artery (Iliff et al., 2007). The authors suggest that nerve-derived EETs may act on the cerebral vasculature (Iliff et al., 2009). In the mouse brain, sEH is immunolocalized in astrocytes rather than neurons in most regions except the central amydgala, where it is primarily expressed in neurons and may play a role in the release of neuropeptides (Marowsky et al., 2009). Thus, it is possible that sEH acts in both VSMCs and nerve cells to regulate blood flow in the brain, through the metabolism of lipid mediators with vasoactive properties or perhaps the ability to trigger the release of neuronal signaling molecules. Elucidation of mechanism will require further examination of regional localization. Supporting a role for sEH outside the regulation of cerebral blood flow, mouse sEH was immunolocalized in

medium and large sized myelinated axons in dorsal root ganglia that are involved in fast nociception and the development of allodynia (Brenneis et al., 2011).

sEH expression in the rat brain is modulated by genetic background and disease states. In rats subjected to middle cerebral artery occlusion, treatment with salvianolic acid A reduced infarct volume and sEH protein expression in the hippocampus, as determined by immunohistochemistry (Wang et al., 2012b). In the SHR, sEH mRNA and protein expression is elevated in the brainstem and hypothalamus compared to the control animals (Sellers et al., 2005). Both these areas are known to be cardioregulatory, adding yet another mechanism by which sEH, through its metabolism of EpFA or other mediators, may be regulating blood pressure. However, Bianco, et al did not detect a change in blood pressure after sEH was overexpressed in the brain (Bianco et al., 2009). Additionally, stroke-prone SHR have lower sEH mRNA expression than stroke-resistant SHR, due to sequence variation in the promoter region of the gene (Corenblum et al., 2008). The change in sEH activity does not result in a significant alteration of plasma EET levels, but the stroke-prone SHR have lower plasma DHET levels. This calls attention to the current lack of knowledge of the biological activity of the DHETs. It also illustrates the differences in lipid metabolism that may occur when sEH is permanently downregulated in an organism.

As in the liver and kidney, male rodents have a higher sEH expression in the brain than females, a result confirmed in primary cortical neurons from both mice and rats, measuring both protein and mRNA levels (Zhang et al., 2009; Fairbanks et al., 2012). Estradiol replacement in ovariectomized (OVX) rats significantly reduces sEH expression in the cerebral cortex, implicating sex hormones in the regulation of sEH protein expression in the brain (Koerner et al., 2008).

It appears that in addition to its well-established role in the regulation of blood flow through metabolism of the EETs, the enzyme may be involved in regulation of the release of neurotransmitters or even memory. This new function may or may not involve the EETs, and will require tools that allow for a finer distinction between the role of sEH in particular brain regions. Because of the potential complications of these overlapping functions of the enzyme, and the possibility that the second, phosphatase activity of the enzyme may be involved, genetic tools will be of particular help.

Lung and Pulmonary Arteries

sEH is immunolocalized to smooth muscle tissue surrounding pulmonary venules in mice, as well as the endothelium of the alveolar capillaries to a lesser degree (Zheng et al., 2001). In humans, sEH protein was identified in lysates prepared from the parenchyma, bronchi, and pulmonary artery (Senouvo et al., 2011). Patients with asthma exhibit a higher expression of sEH in the bronchi, judged by immunohistochemistry (Morin et al., 2010). In contrast, in the rat model of monocrotaline-induced pulmonary hypertension sEH protein levels were decreased in lung tissue (Revermann et al., 2009). Blood flow is regulated in a complex manner in the lung with different regions oxygenated at different stages of the respiratory cycle. This may explain the model and insult-dependent changes in sEH expression. The variation in sEH expression is reminiscent of the brain, where control of both temporal and long term blood flow to certain regions has physiological relevance.

Fat

Fat is a highly structured organ that contains both adipocytes and non-fat stromal cells arranged in a defined architecture. Stromal cells include endothelial cells that form part of the vasculature, as well as inflammatory cells such as macrophages. In mice fed a high fat diet, sEH was immunolocalized in both endothelial and macrophages in adipose tissue, as

would be expected from previous studies (De Taeye et al., 2010). In the high fat diet, 42% rather than 12% of the total calories were derived from fat. Normalized concentrations of sEH mRNA or protein did not change in total fat, but there was variation between sEH expression in perirenal, epididymal, pericardial, and subcutaneous fat pads, indicating specific sEH expression. After immunohistochemistry suggested that sEH was also expressed in adipocytes, the group isolated epididymal fat. sEH mRNA expression was four times higher in the adipocyte-enriched fraction compared to the stromal fraction. Additionally, in cultured 3T3-L1 preadipocytes, differentiation was accompanied with an increase in sEH protein and mRNA expression. As adipose tissue expands in obesity, peripheral resistance increases with tissue mass and vascularization. The observation that sEH increases with adipocyte maturation suggests that anti-inflammatory and anti-hypertensive EpFA will be reduced. Thus one can envision a positive feedback where increased adiposity in turn increases vascular resistance and Ang-II levels, leading to increased sEH levels and decreases in EETs and other EpFA. The presence of sEH in adipocytes suggests a role for sEH beyond the regulation of vascular tone in fat. It is especially intriguing that sEH expression varies between different fat regions. Future studies may discover a role for sEH in the physiological changes that occur within fat in response to chronic disease states. Perhaps sEH expression in fat pads in proximity to the surface of organs such as the kidney or heart will have some relevance to particular disease states such as diabetes or hypertension.

Inflammatory system

sEH is implicated in the regulation of inflammation in several models. Rat alveolar and peritoneal macrophages and peritoneal neutrophils contain detectable amounts of sEH by western blot (Draper and Hammock, 1999). Macrophages isolated from the bone marrow of non-obese diabetic mice and treated with LPS for 12 hours display a greater increase in sEH expression than cells isolated from control mice (Rodriguez and Clare-Salzler, 2006). This indicates that the disease background has a sensitizing effect. sEH protein level was elevated at the graft-vein junction of synthetic arteriovenous grafts in a porcine model, and continued to increase up to 4 weeks post surgery, judged by immunohistochemistry (Sanders et al., 2012). sEH expression appeared to spread beyond the media of the vessel to the adventia by the first week. The authors then cultured human monocytes and confirmed sEH expression by western blot. Finally, in a variety of inflammation-based assays inhibitors of the sEH are more potent at reducing inflammation *in vivo* than NSAIDs and COXIBs and synergize with them (Morisseau and Hammock, 2012; Shen and Hammock, 2012). These results support the view that sEH and by implication EpFA are broadly involved in the regulation of inflammation.

Reproductive system

sEH is expressed in porcine granulosa and theca cells, where protein levels are lower in follicles harvested after the luteinizing hormone surge (Newman et al., 2004). In the mouse, sEH transcript levels in the ovary increase upon stimulation of the estrous cycle, and peak in the leutal phase, when the ovaries contains corpora lutea (Hennebold et al., 2005). The mouse also expresses a splice variant of sEH during the periovulatory phase of a stimulated estrous cycle that has not been identified in other mammals (Hennebold et al., 2005). In the primate corpus luteum, the sEH protein and mRNA levels peak in the late leutal phase of the menstrual cycle (Irusta et al., 2007). The authors suggest that sEH may play a role in either the initiation of corpus luteum formation, and/or in the regression of the structure. In pig, sEH protein increases in the prepubertal cumulus-oocyte complexes (COCs) compared to cyclic females (Paczkowski and Krisher, 2010). Finally, sEH protein is expressed in the human myometrium, as well as the placenta (Corriveau et al., 2009). Taken together, these

results imply that sEH may play a role in fertility, not necessarily limited to the action of the corpus luteum.

In the male reproductive system, sEH protein is expressed in the rat epididymis, specifically the caput, corpus, and cauda (DuTeaux et al., 2004). Interestingly, unlike the liver and kidney, sEH in the rat epididymis is not induced by clofibrate treatment. This observation fits the pattern that sEH induction by PPARα and PPARγ ligands varies among organs.

The difference between sEH expression between the sexes, as well as modulation of sEH expression by estrogens show that careful attention should be paid to the sex of the animals chosen for *in vivo* experiments. In the case of estrogens, this is a further example of receptor-dependent regulation of sEH, and the enzyme's presence at specific moments during the fertility cycle indicate a specific, and possibly novel role for sEH and EpFA in reproduction.

Neoplasms and tumors

A 2006 study examined the expression of human sEH in 15 different neoplastic tissues (Enayetallah et al., 2006b). The authors noticed a significant reduction in sEH protein expression compared to healthy tissue in renal and liver malignant neoplasms, but an increase in other cancers, such as advanced ovarian cancer. This reduction in sEH expression was detected in the tissue surrounding the neoplasms. sEH protein expression is reduced in a mouse Lewis lung carcinoma model, where sEH protein levels are reduced in tumor endothelial cells versus normal endothelial cells, as well as in tumor lysates from large versus small tumors (Panigrahy et al., 2012). sEH protein expression is also reduced in melanoma liver metastases versus healthy liver (Panigrahy et al., 2012). These data indicate that sEH expression is increased or decreased in specific cancers. Given the complex process of tumor initiation, growth and metastasis, more work will be required to elucidate the role of sEH in cancer. Questions will include how sEH expression affects the vascularization of larger tumors, as well as the role the enzyme plays in preneoplastic lesions and surrounding neoplasms that eventually develop into tumors.

8. sEH genetic models

The first sEH knockout mouse colony was created by disruption of exon one by a Neo cassette (Sinal et al., 2000). Early generations of these sEH null mice displayed lower resting blood pressure than wild-type, though females displayed no change in blood pressure. Later, this strain was backcrossed into C57Black/6 genetic background, and a new colony established by embryo transfer (Luria et al., 2007). There was no difference in resting blood pressure between sEH null mice from the new colony and wild-type mice. An independent sEH null colony engineered by Boehringer Ingelheim also did not display a change in blood pressure, raising the question of compensatory mechanisms in the knockout animals (Luria et al., 2007). The authors did not detect sEH protein expression in the liver, kidney, or heart from animals of either the Boehringer or the backcrossed colony and homogenates generated from liver and kidney displayed an increase in EpFA. However, the vasoconstrictive and hypertensive ω-hydroxylase metabolite, 20-HETE (see Figure 4) was enhanced 4-fold in kidney lysates. When the plasma lipids were analyzed, the EpFA were increased and the corresponding diols were decreased and there was no change in lipoxygenase- and cyclooxygenase-dependent oxylipins. Other differences between the sEH null mice and wild-type animals include variation in plasma testosterone, sperm count and testicular size in males and a decrease in plasma cholesterol levels (Luria et al., 2009). Adenosine-induced relaxation of coronary artery rings isolated from knockout animals depends on the upregulation or downregulation of several receptors compared to the wild-type mice, including A_{2A} AR, A_1 AR, PPARγ, and PPARα (Nayeem et al., 2013). This

indicates that compensatory mechanisms in the null mice involve complex changes in signaling pathways relating to the maintenance of vascular tone. These knockout strains, particularly when coupled with chemical knockouts using sEH inhibitors and EET antagonists have proven very valuable in elucidating the physiological role of sEH. Some important findings are summarized in Table 2. Hopefully, conditional, tissue-selective and other modifications will provide even greater insight in the future.

Transgenic mice that overexpress human sEH in the endothelium were produced by inserting sEH cDNA under the control of a Tie2 promoter (Edin et al., 2011). Tie2-sEH Tr mice have a reduced plasma epoxide-to-diol ratios of 14,15 EET and 12,13-epoxyoctadecenoic acid and do not exhibit decreased infarct size or improved heart function after ischemia/reperfusion (Edin et al., 2011). Aortic endothelial cells isolated from Tie2-sEH Tr mice display reduced migration compared to wild-type cells (Panigrahy et al., 2012).

Acute Inflammation and Sepsis

The injection of lipopolysaccharide (LPS), a fragment from the bacterial wall, is an established model of acute inflammation and septic shock. Recovery from acute inflammation induced by an intraperitoneal LPS injection of 10 mg/kg was enhanced in the null relative to the wild-type, as assessed by a return of blood pressure to pre-treatment levels (Luria et al., 2007). This was reflected in a higher survival rate: 100% of the sEH null mice survived the challenge while 18% of the wild-type mice died within 24 hours. Recovery of healthy blood pressure in the null animals is enhanced by treatment with aspirin or the 5-lipoxygenase activation protein (FLAP) inhibitor MK886 (Liu et al., 2010). Aspirin or MK886 treatment does not improve the recovery of wild-type animals, indicating that the sEH null background increases the anti-inflammatory effect of these compounds. However, treatment with indomethicin does not produce an additive anti-inflammatory effect (Oni-Orisan et al., 2012). sEH null mice display a reduction in nuclear factor (NF)-κB signaling compared to controls after 40 mg/kg LPS injection (Deng et al., 2011). They also display reduced cellular adhesion molecule, chemokine and cytokine expression, as well as a reduction in neutrophil infiltration in lungs. sEH null mice injected with a lower dose of LPS (1mg/kg) did not display a reduction in inflammatory gene expression in the liver compared to controls (Fife et al., 2008). In general, these results support the view that sEH null animals have reduced acute inflammatory response and/or enhanced recovery from LPS challenge.

Ischemia/Reperfusion Injury in the Heart and Brain

When isolated hearts in a Langendorff apparatus are subjected to ischemia and reflow, hearts from sEH null mice display enhanced recovery of left ventricular developed pressure compared to controls (Seubert et al., 2006; Chaudhary et al., 2012). In an *in vivo* model where the left coronary artery was surgically occluded and then released from its snare to allow reperfusion, the infarction area was reduced in sEH null animals, indicating reduced injury (Motoki et al., 2008). However, in an *in vivo* mouse model of cardiac arrest followed by cardiopulmonary resuscitation (CPR), the knockouts displayed a longer time before restoration of spontaneous circulation during CPR and a decreased survival rate (Hutchens et al., 2008). This may reflect the altered blood pressure regulation in the knockout mouse reported by Luria, et al as well as the complex relationship between sEH function in the vasculature and in heart muscle cells (Luria et al., 2007).

The knockout animals display less tissue damage and enhanced recovery in cerebral ischemia/reperfusion injury and acute brain trauma models. sEH null animals subjected to a single round of middle cerebral occlusion followed by reperfusion have a smaller infarct size (Zhang et al., 2008b). Cerebral blood flow rates are also increased in the knockout animals,

mirroring the results in heart (Zhang et al., 2008b). Interestingly, the authors report no difference in free 14,15-EET levels between knockout and wild-type brains. This highlights the diverse roles of EET signaling in specific organs and disease states, and cautions against making generalizations about the sEH knockout animals based on plasma levels of EpFA. Compensatory mechanisms may operate differently in different tissues and produce localized effects not reflected systemically. When optical micro-angiography was used to monitor revascularization in mice that had undergone a traumatic brain injury, the sEH null mice exhibited accelerated revascularization and enhanced blood volume at the lesion site one month after the injury (Jia et al., 2011). These data indicate that repair of the tissue is due to revascularization after the injury, rather than a decrease in the damage or immediate repair after injury. sEH null mice display improved motor coordination on a beam walk task but an impaired performance in a Morris water maze test compared to wild-type mice before and after traumatic brain injury induced by unilateral controlled cortical impact (Strauss et al., 2012). A promising tool for the further investigation of the role of sEH in the brain is a transgenic mouse model with neuronal over-expression of sEH created by fusing the rat synapsin 1 promoter to a mouse sEH cDNA (Bianco et al., 2009).

Inflammatory Bowel Disease

The NSAID piroxicam synergizes the development of inflammatory bowel disease (IBD) in IL-10(-/-) mice (Zhang et al., 2012c). A reduction in inflammatory cytokines was noted in IL-10(-/-)/sEH(-/-) animals relative the IL-10(-/-) mice after prioxicam treatment. There was also less ulcer formation and infiltration of inflammatory cells in the bowels of the IL-10(-/-)/sEH(-/-) animals. In this model, the eicosanoid profile, including several metabolites from all three branches of the ARA cascade, was shifted away from propagation of inflammation. Null mice displayed a lower tumor multiplicity, tumor size, and a decrease in precancerous dysplasia (Zhang et al., 2012b). The NSAID-induced gastrointestinal ulcers were also reduced in this model, an important observation, particularly for individuals who must use high doses of NSAIDs for extended periods. However, humans appear quite resistant to NSAID-induced gastrointestinal lesions compared to canine, equine, and feline patients. An increase in the EET-to-diol ratio, including 14,15-EET, was noted in the plasma of the knockout and IL-10(-/-)/sEH(-/-) animals compared to the IL-10(-/-) mice.

Diabetes

The sEH knockouts have improved function in a streptozotocin (STZ)-induced model of diabetes (Luo et al., 2010). They do not suffer from hyperglycemia, and have improved insulin secretion and glucose tolerance. The islet cells in the sEH knockout animals exhibit an increase in glucose-stimulated insulin secretion (GSIS) and intracellular calcium levels. There is also a decrease in islet cell apoptosis. This anti-apoptotic effect may contribute to the organ protection observed in brain, heart and kidney. Supporting enhanced organ protection in the nulls, knockout mice display a decrease in urinary markers of kidney inflammation and damage such as albumin and monocyte chemoattractant protein-1 in the STZ model (Elmarakby et al., 2011). This protective effect occurs in kidney tubules, in addition to the pancreas. The sEH knockout animals exhibit decreased renal tubular apoptosis when treated with STZ (Chen et al., 2012). These last two papers report an increase in urinary EET over DHET relative to the wild-type controls. The organ protective effect observed in the sEH knockout animals extends beyond the STZ models. In a model of type-II diabetes where mice are fed a high fat diet, the knockout animals display larger islets, increased pancreatic vasculature, and improved insulin signaling (Luria et al., 2011). In the STZ and the type-II high fat diet models, the sEH knockout animals have improved islet function, as well as an increased resistance to islet cell apoptosis, implicating EpFA in the protection of the kidney and pancreas.

Pulmonary Hypertension

sEH is implicated in the pathology of pulmonary hypertension (Keseru et al., 2010). When the sEH null mice are subjected to chronic hypoxia, they develop a more pronounced right heart hypertrophy, enhanced pulmonary artery muscularization, and decreased exercise performance compared to the wild-type animals. Interestingly, long term treatment with compounds that inhibit the epoxide hydrolase activity does not duplicate the phenotype observed in the knockout mice, raising the possibility that the phosphatase activity of the enzyme is important to the development or progression of pulmonary hypertension. This is a reminder that the knockout sEH animals are deficient in both epoxide hydrolase and phosphatase activities, and that examination of the difference in phenotypes obtained with inhibitors and the knockout animals may give some insight into the function of the sEH phosphatase activity. There is a need for future knockout and knock-in models where the sEH and phosphatase catalytic activities are dealt with separately and the possible structural roles of the sEH protein are treated apart from the catalytic roles.

9. Conclusion

This review has not fully addressed the use of substrates to distinguish sEH from microsomal EH. Interested readers are referred to historical reviews (Morisseau and Hammock, 2008; Imig, 2012). Also, this review has not discussed sEH inhibition. sEH inhibitors are a powerful tools for the elucidation of sEH function and are being pursued as possible therapies for diseases such as hypertension. Readers interested in the history of the development of sEH inhibitors are referred to several recent reviews (Shen, 2010; Morisseau and Hammock, 2012; Shen and Hammock, 2012) and included references.

The localization of sEH in the VSMCs and endothelial cells in several tissues is consistent with its proposed role in the metabolism in the vasoactive EETs. By converting EETs to their corresponding diols, sEH can exert a regulatory effect on vascular tone and systemic blood pressure or create regional differences in blood flow in organs such as the lung and brain. Similarly, the localization of sEH in macrophages and other inflammatory cells, as well as the increase in sEH expression in the region of grafts is consistent with its role in the regulation of inflammation. The interesting localization of sEH in specific brain regions or the tissues of the reproductive system or fat cells may indicate as yet undiscovered functions for sEH that go beyond the maintenance of blood pressure and regulation of inflammation. Such roles could involve pain, cognition and other neurological processes as well as reproductive processes ranging from ovulation to birth.

The sEH knockout mice are valuable models that have associated the sEH protein with disease states and physiological function. The apparent adjustment to the genetic elimination of the sEH does complicate interpretation of results, however. Additionally, sEH contains two active sites with two different catalytic activities. For this reason, comparison of the knockouts to inhibition studies that use sEH epoxide hydrolase inhibitors must be made carefully. Also, the complete elimination of the sEH activity throughout the life of the mouse is expected to generate different biological effects than the transient and partial inhibition of the enzyme with pharmaceutical probes. Greater insight can be anticipated from tissue selective and temporally dependent genetic alterations in sEH activity in the future. Combining these genetic tools with pharmacological inhibitors of the sEH and mimics of EpFA that function as stable agonists and antagonists will provide still greater insight.

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Abbreviations

20-HETE	20-hydroxyeicosatetraenoic acid
Ang-II	angiotensin II
AP-1	activator protein 1
ARIC	atherosclerosis risk in communities
ATF-6	activating transcription factor-6
CAC	coronary artery calcification
CARDIA	coronary artery risk development in young adults
ChIP	chromatin immunoprecipitation
CPR	cardiopulmonary resuscitation
DHA	docosahexaenoic acid
DHETs	dihydroxyeicosatrienoic acids
EETs	epoxyeicosatrienoic acids
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid
EpFA	epoxy-fatty acids
FLAP	5-lipoxygenase activation protein
GSIS	glucose-stimulated insulin secretion
Hcy	homocysteine
HUVECs	human umbilical vein endothelial cells
IBD	inflammatory bowel disease
IL-10	interleukin-10
LPS	lipopolysaccharide
NSAID	nonsteroidal anti-inflammatory drug
OVX	ovariectomized
PPAR	peroxisome-proliferator activated receptors
RAAS	renin-angiotensin aldosterone system
RBC	red blood cell
sEH	soluble epoxide hydrolase
SHR	spontaneously hypertensive rat
SNP	single nucleotide polymorphisms
SP-1	specificity protein 1
STZ	streptozotocin
THF	tetrahydrofuran
UPRE	unfolded protein response elements
UTR	untranslated region

VSM	vascular smooth muscle
WKY	Wistar-Kyoto

Highlights

- Soluble Epoxide Hydrolase (sEH) mammalian gene structure
- sEH single nucleotide polymorphisms
- Expression of sEH in mammals
- sEH knockout mice and disease models

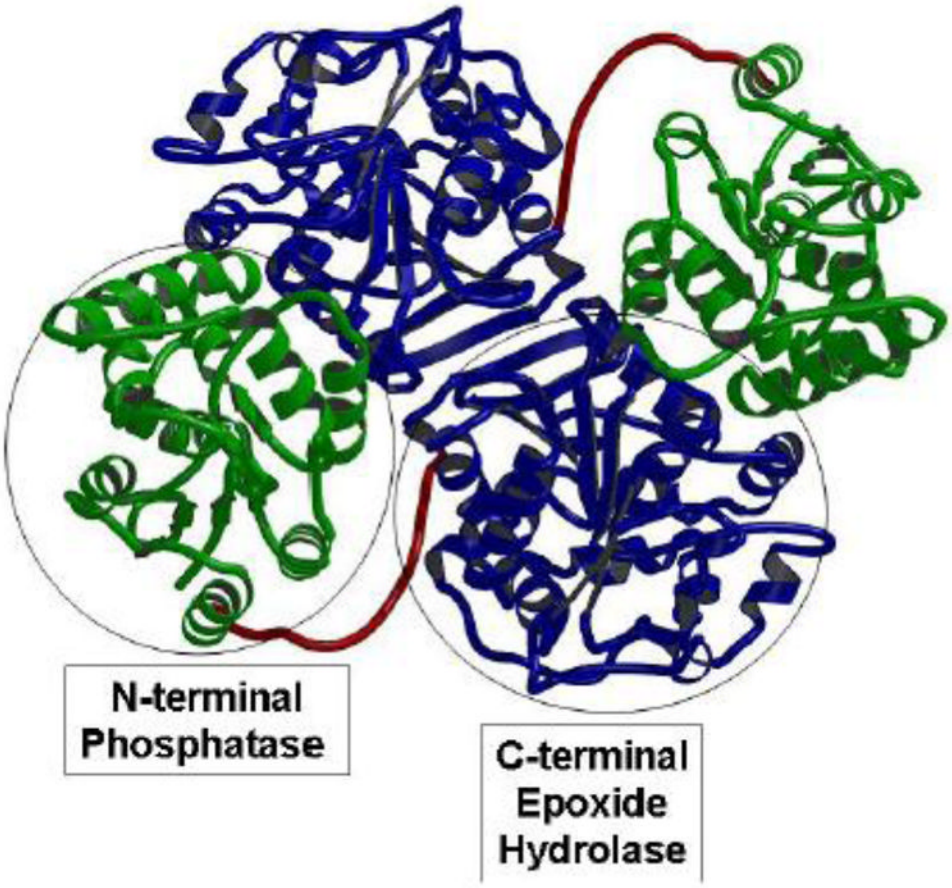

Figure 1.
Crystal structure of the sEH dimer (PDB accession code 1S80 (Gomez et al., 2004).) The sEH monomer is composed of two globular regions displaying alpha/beta fold tertiary structure connected by a short proline-rich linker. The sEH dimer is anti-parallel, so that the N-terminal region of one monomer is in contact with the C-terminal region of the other. The catalytic site for the epoxide hydrolase activity is located within the C-terminal region, while the phosphatase activity is located within the N-terminal region.

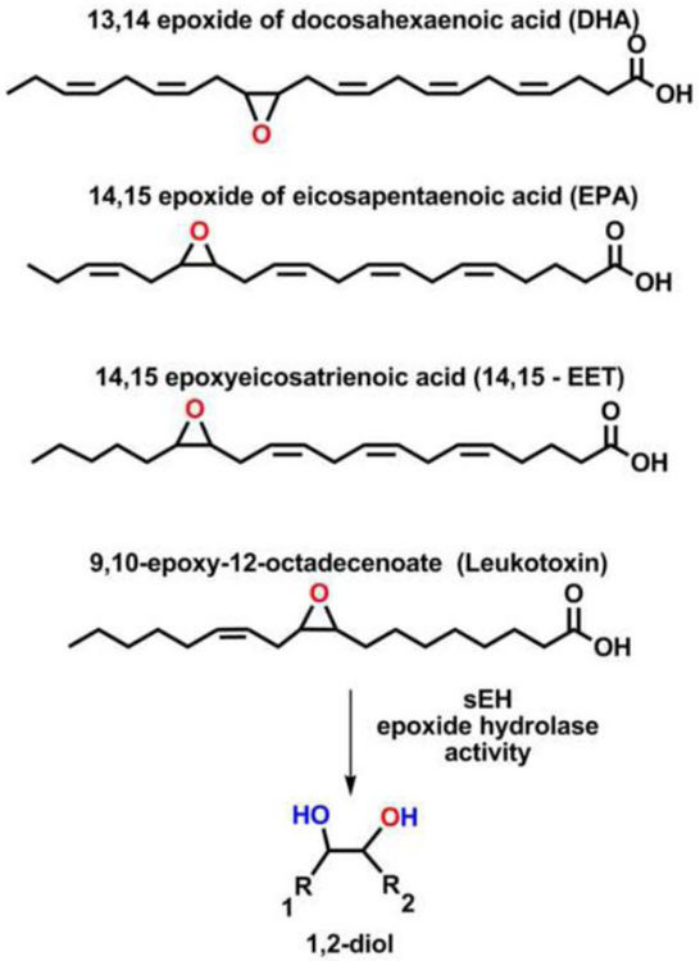

Figure 2.

Substrates and products of sEH epoxide hydrolase activity. Through the addition of water, sEH converts lipid epoxides to diols (the oxygen of the epoxide moiety is in red). Potential substrates for sEH include the omega-three lipid epoxides formed from DHA and EPA, the omega-six lipid epoxides formed from arachidonic acid (ARA), linoleic acid, and stearic acid. The regioisomers preferred by the human sEH are displayed (Morisseau et al., 2010).

	Caenorhabditis elegans	Unknown Species	*Strongylocentrotus purpuratus* *SPEH1*	*SPEH2*	*Gallus gallus*	*Homo sapiens*
Phosphatase	Unknown		No	No	No	Yes
Epoxide Hydrolase	Yes		Yes	No	Yes	Yes

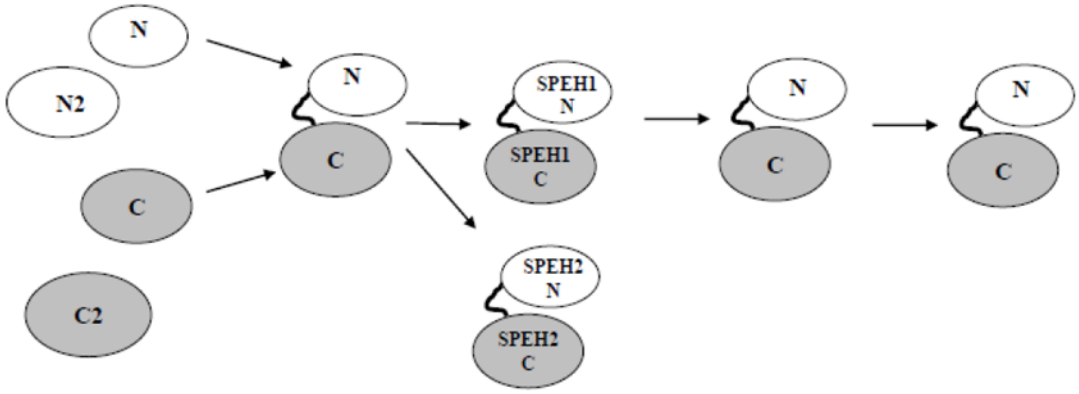

Figure 3.

sEH evolution. The mammalian sEH is the product of a gene fusion event and subsequent gene duplication that resulted in two full length sEH genes in *S. purpuratus*. One of these genes produces a protein with no phosphatase or epoxide hydrolase activity, while the other produces a protein with epoxide hydrolase but no phosphatase activity. In chicken there is a single sEH homolog with epoxide hydrolase but no phosphatase activity. The mammalian sEH in mouse, rat, pig, and human exhibit both epoxide hydrolase and phosphatase activities.

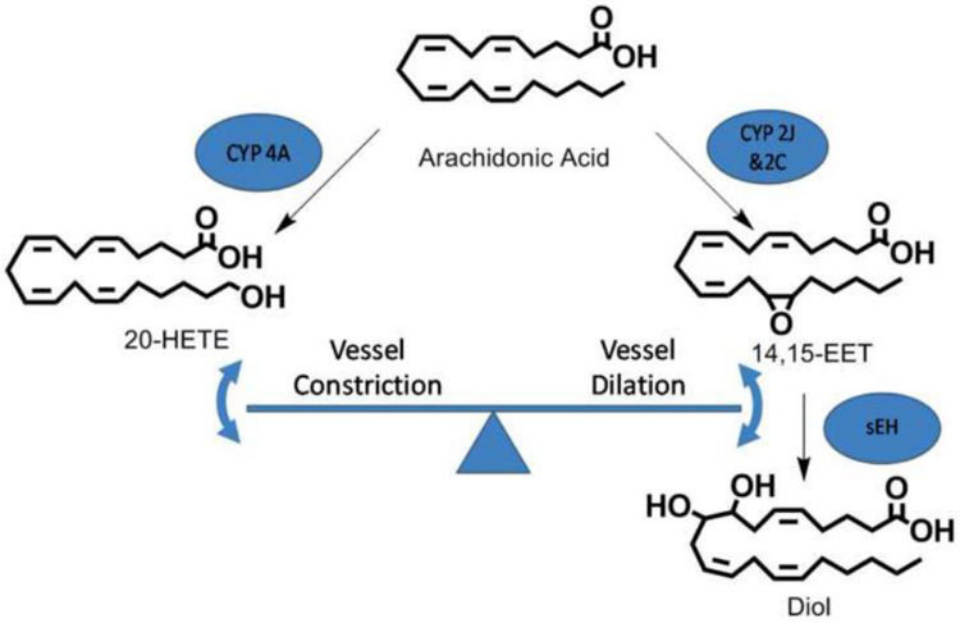

Figure 4.

Simplified model of the balance of lipid mediators in the kidney. The vasodilatory epoxyeicosatrienoic acids (EETs—represented by the single isomer 14,15-EET here) are produced from arachidonic acid (ARA) through the action of cytochrome P450s (CYPs) 2J and 2C. This anti-hypertensive signal is offset by a second metabolite of ARA, 20-hydroxyeicosatetraenoic acid (20-HETE) produced by CYP 4A. The balance of antihypertensive and hypertensive signals is partially maintained through the action of sEH. sEH metabolizes the EETs to diol species that are readily conjugated and removed from the site of action in the kidney vasculature. When sEH is inhibited there is an increase in EET levels relative to 20-HETE levels. This tips the balance in favor of vasodilation, creating an anti-hypertensive effect. The process is highly simplified since the epoxy fatty acids and their corresponding diols can be of multiple chain lengths in both the ω-3 and ω-6 series with varying regio, geometrical, and optical isomers involved.

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Table 1

Modulation of sEH expression in selected tissues

Ref	Organ/Cell	Localization	Species	Conditions	Result	Detection method
(Zhang et al., 2012a)	HUVECs		Human	Hcy treatment	Upregulation	Western qRT-PCR
(Zhao et al., 2005)	Vasculature	Mesenteric Artery	Rat	Lean vs. obese Zucker	Upregulation	Western qRT-PCR
(Mark-Kappeler et al., 2011)	Vasculature	Mesenteric Artery	Rat	Ethinyl estradiol, tamoxifen, and raloxifene-treated ovariectomized animals	Upregulation	cDNA microarray
(Zhang et al., 2012a)	Aorta	Intima	Mouse	Hcy treatment	Upregulation	qRT-PCR
(Maresh et al., 2005)	Aorta	Endothelium	Mouse	Exposure to cigarette smoke	Upregulation	qRT-PCR
(Gao et al., 2011)	Coronary artery		Mouse	Renal artery stenosis	Upregulation	Western
(Aboutabl et al., 2009)	Heart		Rat	3-methylcholanthrene and benzo(a)pyrene treatment	Upregulation	Western qRT-PCR
(Xu et al., 2006)	Heart	Atrial and ventricular myocytes	Mouse	Thoracic aortic constriction	No change	Western qRT-PCR
(Zordoky et al., 2008)	Heart		Mouse	Isoproterenol treatment	Upregulation	qRT-PCR
(Ai et al., 2009)	Heart	Left ventricle	Rat	SHR vs. Wistar control	Upregulation	Western
(Ai et al., 2009)	Heart	Left ventricle	Rat	Ang-II treatment	Upregulation	Western
(Ai et al., 2009)	Heart	Left ventricle	Rat	Exercise training	No change	Western
(Ai et al., 2009)	Heart	Left ventricle	Rat	Norepinephrine treatment	No change	Western
(Yu et al., 2000)	Kidney	Cortex	Rat		Upregulation	Western Northern
(Seubert et al., 2005)	Kidney		Rat	SHR vs. Wistar control	Upregulation	cDNA microarray Northern
(Koeners et al., 2011)	Kidney		Rat	SHR vs. Wistar control	Upregulation	qRT-PCR
(Imig et al., 2002)	Kidney	Cortex	Rat	Ang-II infusion	Upregulation	Western
(Zhao et al., 2004)	Kidney	Cortex	Rat	Ang-II infusion	Upregulation	Western
(Zhao et al., 2004)	Kidney	Microvessels	Rat	Ang-II infusion	Upregulation	Western
(Walkowska et al., 2010)	Kidney	Cortex	Rat	Renal artery stenosis	Upregulation	Western
(Jung et al., 2010)	Kidney		Mouse	5/6 nephrectomy	Downregulation	Western qRT-PCR
(Oguro et al., 2009)	Kidney		Mouse	Streptozotocin treatment	Downregulation	Western qRT-PCR
(Oguro et al., 2009)	Liver		Mouse	Streptozotocin treatment	Downregulation	Western
(Fife et al., 2008)	Liver		Mouse	LPS treatment	Downregulation	qRT-PCR
(Sellers et al., 2005)	Brain	Brain stem Hypothalamus	Rat	SHR vs. Wistar control	Upregulation	Western qRT-PCR

Ref	Organ/Cell	Localization	Species	Conditions	Result	Detection method
(Koerner et al., 2008)	Brain	Cortex	Rat	Estradiol treatment of ovariectomized animals	Downregulation	Western
(Revermann et al., 2009)	Lung		Rat	Monocrotaline treatment	Downregulation	Western
(Keseru et al., 2010)	Lung		Human	Hypoxia	Dowregulation	Western qRT-PCR
(Morin et al., 2010)	Lung	Bronchi	Human	Asthma vs. control	Upregulation	Immuno histochem
(Rodriguez and Clare-Salzler, 2006)	Macrophages	Bone marrow derived	Rat	LPS treatment of non-obese diabetic animals	Upregulation	qRT-PCR

Table 2

sEH null mice and disease models

Ref	Disease	Model	Summary of physiological differences compared to wild-type animals
(Luria et al., 2007; Fife et al., 2008; Liu et al., 2010; Deng et al., 2011)	Acute Inflammation	LPS-induced	Increased survival Reduced inflammatory cytokine and chemokine expression Enhanced return to basal blood pressure (BP) from hypotensive state
(Seubert et al., 2006)	Cardiac Ischemia/Reperfusion Injury	Isolated heart in Langendorff apparatus	Enhanced recovery of LVDP Reduced infarct size
(Motoki et al., 2008)	Cardiac Ischemia/Reperfusion Injury	Surgical occlusion	Reduced infarct size
(Hutchens et al., 2008)	Cardiac arrest	Cardiac arrest and CPR	Longer time to recover BP Reduced survival
(Zhang et al., 2008)	Cerebral Ischemia/Reperfusion Injury	Middle cerebral artery occlusion/ reperfusion	Smaller infarct Increased cerebral blood flow
(Jia et al., 2011)	Brain Trauma		Increased blood volume at the lesion site one month after injury Increased rate of revascularization
(Zhang et al., 2012a; Zhang et al., 2012b)	Inflammatory Bowel Disease	sEH (-/-)/IL-10 (-/-) fed piroxicam vs. IL-10 (-/-) mice fed piroxicam	Reduction in inflammatory cytokines Decreased ulcer formation Decreased inflammatory cell infiltration in the bowel Reduced number and size of tumors
(Luo et al., 2010)	Diabetes	Streptozotocin (STZ)-induced	Improved glucose tolerance Improved insulin secretion Reduction in islet apoptosis
(Elmarakby et al., 2011)	Diabetes	STZ-induced	Reduced renal inflammation and damage
(Chen et al., 2012)	Diabetes	STZ-induced	Reduced renal tubular apoptosis
(Luria et al., 2011)	Diabetes	Type II high fat dietary model	Increased islet size and pancreatic vascularization Improved insulin signaling
(Keseru et al., 2010)	Pulmonary Hypertension	Hypoxia-induced	Increased right heart hypertrophy Enhanced pulmonary artery muscularization Decreased exercise performance
(Manhiani et al., 2009)	Hypertension	Deoxycorticosterone acetate treatment plus high salt diet (DOCA-salt)	Reduction in mean arterial BP Reduction in urinary MCP-1 excretion

Ref	Disease	Model	Summary of physiological differences compared to wild-type animals
			Reduced macrophage infiltration Reduced albuminuria
(Revermann et al., 2010)	Arteriosclerosis	Femoral cuff in Apo(-/-) animals	Reduction of neointima formation Reduction in proinflammatory gene expression in femoral arteries
(Hercule et al., 2009)	Hypertension	L-NAME induced	No difference in BP and EDHF responses
(Inceoglu et al., 2012)	Niacin flushing		Reduction in flushing
(Liu et al., 2012)	Kidney damage	Cisplatin-induced	Reduction in serum urea nitrogen and creatinine Reduction in renal tubular damage Reduced neutrophil infiltration
(Sander et al., 2012)	Wound healing	Dermal wound on ear	Accelerated wound closure