Soluble Epoxide Hydrolase: A Novel Target for the Treatment of Hypertension

Xiang Fang*

Department of Biochemistry, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA

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Abstract: Epoxide hydrolases are a group of enzymes that convert the epoxide group of chemical compounds to corresponding diols by the addition of water. Soluble epoxide hydrolase (sEH, formerly referred to as cytosolic epoxide hydrolase), which is widely distributed in mammalian tissues, is the primary enzyme responsible for the conversion of epoxyeicosatrienoic acids (EETs), the bioactive lipid mediators formed from arachidonic acid by cytochrome P450 epoxygenase, to their corresponding diols. EETs, but not their diols, are endogenous anti-hypertensive eicosanoids. Disruption of the sEH gene in male mice decreases blood pressure, and inhibition of sEH decreases blood pressure in several experimental hypertensive models. Potent selective sEH inhibitors have been developed, and these sEH inhibitors have potential to become a novel class of anti-hypertensive drug.

Keywords: Soluble epoxide hydrolase, inhibitors, arachidonic acid, cytochrome P450, epoxyeicosatrienoic acids, hypertension, vascular tone.

BACKGROUND

Eicosanoids, which are derived from arachidonic acid (AA), are endogenous bioactive lipid mediators that play important roles in regulating homeostasis and many pathophysiological processes. These eicosanoids are formed through the action of three classes of metabolic enzymes: cyclooxygenase, lipoxygenase, and cytochrome P450 (CYP). AA is converted to epoxyeicosatrienoic acids (EETs) by CYP epoxygenases [1-3]. EETs are considered to be endogenous anti-hypertensive eicosanoids. Pharmacological interventions to either increase EETs synthesis or prevent degradation of EETs have been proposed as new approaches for the treatment of hypertension. Because soluble epoxide hydrolase (sEH) is the main enzyme responsible for conversion of EETs to their corresponding dihydroxyeicosatrienoic acids (DHETs), which are biologically inactive in many systems, sEH has become a novel target for the treatment of hypertension. Recently, several patents related to sEH inhibitors and their potential applications for the treatment of hypertension have been issued by US Patent and Trademark Office [4-7].

EPOXYEICOSATRIENOIC ACIDS (EET)

1. Synthesis & Metabolism

CYP epoxygenases, primarily the CYP2C and 2J isoforms in humans, produce four EET regioisomers from arachidonic acid; 5,6-, 8,9-, 11,12-, and 14,15-EET. Fig. **1** illustrates the synthesis of 11,12-EET from arachidonic acid and its conversion to 11,12-DHET. EETs and/or DHETs are detected in blood, urine, and various tissues such as heart, kidney, liver, and brain [1-3]. 11,12-EET, and 14,15-EET are the predominant EETs produced by many different cells and tissues. In the coronary circulation, CPY 2C is an endothelium-derived hyperpolarizing factor (EDHF) synthase, and the main CPY 2C product is 11,12-EET [8]. Increased production of the EETs are observed when cells were stimulated by bradykinin, shear stress, or ischemia/reperfusion [9, 10]. CYP epoxygenase 2C is an inducible enzyme. Prolonged cyclic stretch (6%, 1 Hz, 10 minutes, 4 to 36 hours) increases the expression of CYP 2C mRNA and protein 5- to 10-fold that is accompanied by a 4- to 8-fold increase in EET production [11].

Endogenous EETs can be stored in esterified form in phosphoglycerides. These EETs are esterified at the *sn*-2 position of the glycerol moiety [12], and they can be released upon stimulation [3]. Furthermore, cells rapidly take up radiolabelled EETs when they are added to the culture medium. Vascular cells have relative large capacity to take up EETs; for example, the uptake of 14,15-EET was not saturated when smooth muscle cells were incubated with 20 μM 14,15-EET [13]. The comparative uptake by vascular cells is 8,9- >11,12- >14,15-EET [13, 14]. In endothelial cells, most of the EETs available in the extracellular medium are incorporated into phospholipids at *sn*-2 position, whereas a sizable amount of the available EETs is esterified into *sn*-1 position in smooth muscle cells [3, 15].

EETs are enzymatically hydrated to their corresponding diols by sEH in mammalian tissues and cells as illustrated for 11,12-EET in (Fig. **1**). The conversion of EET to the corresponding DHET by sEH is regioselective. 14,15-EET is the preferred substrate as compared with 11,12- and 8,9-EET. Regioselective and/or enantioselective oxirane water addition occurs during formation of asymmetric 14,15-, and 8,9-DHET. 14(*R*),15(*S*)-, 11(*S*),12(*R*)-, and 8(*S*),9(*R*)-EET are metabolized at substantially higher rates than their antipodes. Conversion of EETs to methyl esters decreases the rates of epoxide hydration, and catalytic hydrogenation of EETs also reduces the rates of hydration [16, 17]. In

*Address correspondence to this author at the Department of Biochemistry, University of Iowa, Iowa City, IA 52242; Fax: 319 335-9570; E-mail: xiang-fang@uiowa.edu

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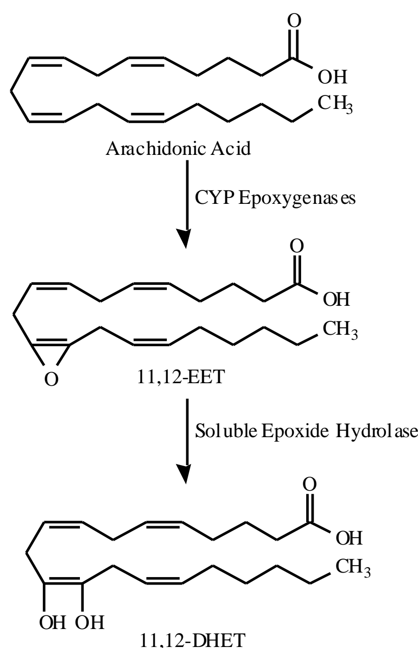

Fig. (1). Synthesis and metabolism of 11,12-EET.

addition to conversion to DHETs, EETs are converted to chain-shortened, beta-oxidized epoxy fatty acids that are predominately released into the medium. They also are converted to the elongated epoxy fatty acids that are predominately retained by the cells [13, 18-20].

2. Biological Activities

EETs have a number of functional effects, particularly in cardiovascular system. EETs produce vasodilation in various vascular beds such as the renal, coronary, intestinal, and cerebral circulation [1-3], and this has been attributed to activation of Ca^{2+} -activated K^+ channels in the smooth muscle (BK_{Ca}). A substantial amount of evidence suggests that EETs may function as EDHF in different vascular beds [8, 21]. For example, EETs relaxes precontracted coronary vessels, and rasorelaxation induced by EETs is blocked by tetraethylammonium, an inhibitor of BK_{Ca}, charybdotoxin, and high K^+. Furthermore, EETs increase the open-state probability of a BK_{Ca} in coronary smooth muscle cells [21]. EETs inhibit Na^+ transport in the proximal tubule and collecting duct of the kidney [22], and they also inhibit the renal Na^+/K^+-ATPase [23]. Furthermore, EETs inhibit 86Rb uptake in renal epithelial cells [24], and they modulate smooth muscle migration [25], prostaglandin E_2 production [26], and aromatase activity [27]. EETs also inhibit platelet aggregation and Ca^{2+} entry induced by AA, thrombin and thapsigargin [28, 29]. In the endothelium, EETs inhibit cytokine-induced inflammatory responses [30], increase Ca^{2+} entry [31], enhance fibrinolysis [32], and stimulate tube formation [33]. These biological activities are regulated by metabolism of the EETs. Conversion of EETs to DHETs by sEH has been generally considered to be an inactivation process. DHETs have diminished activity in many systems as compared with the corresponding EET, including relaxation of the preglomerular vasculature [34], inhibition of prostaglandin formation [26], calcium mobilization [35], stimulation of ADP-ribosylation [36], and relaxation of the bovine coronary artery [37]. Therefore, inhibition of sEH to increase the concentration of EETs might have some beneficial effects in the cardiovascular system.

MAMMALIAN SOLUBLE EPOXIDE HYDROLASE

1. Distribution & Characteristics

Epoxide hydrolases (EC 3.3.2.3) are a group of enzymes that convert the epoxide group of chemical compounds to corresponding diols by the addition of water. Based on the structure of their substrates, localization of the enzymes, and species, six distinct epoxide hydrolase sub-types have been identified. These include the mammalian sEH, plant sEH, the hepoxilin hydrolase, leukotriene A_4 hydrolase, the microsomal epoxide hydrolase, and the insect juvenile hormone epoxide hydrolase [38-40]. Mammalian sEH is one of the most important subtypes in EH superfamily and is responsible for epoxy fatty acid metabolism. High sEH activity has been detected in liver, kidney, and vascular tissues (3), and human sEH from liver and kidney has been purified. EPHX2, the gene encoding sEH, has been cloned and characterized [41]. EPHX2 is located in chromosomal region 8p21-p12 [42]. It is approximately 45 kb in length and consists of 19 exons with lengths from 27 to 265 bp [43].

Human sEH is genetically polymorphic. Eight variant cDNA loci from 25 human livers have been detected. The coding region contains five silent single nucleotide polymorphisms and two variant loci resulting in altered protein sequences [Arg^{287} to gln^{287} (exon 8); Arg^{403} to $Arg^{403-404}$ (exon 13) [44]. Thirty-six single nucleotide polymorphisms have been identified in the EPHX2 gene in Japanese individuals, as well as one insertion/deletion polymorphism in the 5' flanking region [45]. These variants of the sEH gene may explain previously reported differences in sEH activity among these individuals [46].

2. Enzymatic Mechanisms

Epoxide hydrolases are members of the / hydrolase fold family. These enzymes hydrolyze their substrates in a two-step reaction involving the formation and hydrolysis of a covalent acyl- or alkyl-enzyme intermediate [47, 48]. Similar to other / hydrolase fold enzymes, the Asp^{333} and His^{523} residues that are required for the catalytic activity of sEH are conserved in all epoxide hydrolases. No activity was detectable after the replacement of Asp^{333} by Ser, and mutation of His^{523} to Gln produced greater than 99 % loss of sEH specific activity [49]. Site-directed mutagenesis studies indicate that Asp^{495} also is required for activity of sEH, and it appears that Asp^{333}, Asp^{495} and His^{523} form the catalytic triad [48]. Furthermore, the crystal structure of mouse sEH suggests that Tyr^{381} and Tyr^{465} can act as acid catalysts, activating the epoxide ring and facilitating the formation of a covalent intermediate between the epoxide and the enzyme [50].

The mammalian sEH is a homodimer, and each subunit contains carboxyl- and amino-terminal domains. The active site for epoxide hydrolysis is located in the carboxyl-terminal domain; The amino-terminal domain of sEH appears to play a critical structural role by stabilizing the

dimer in a distinctive conformation [51]. Furthermore, the amino-terminal domain of sEH also has phosphatase activity [52]. Phosphorylated hydroxy- lipids are excellent substrates for the human sEH, particularly the monophosphate of dihydroxy stearic acid [53].

sEH INHIBITORS

Several generations of sEH inhibitors have been developed. The early compounds include substituted chalcone oxides and *trans*-3-phenylglycidols, two classes of structurally similar (both are epoxides) sEH inhibitors [54, 55]. Substituted chalcone oxides are more potent than the *trans*-3-phenylglycidols. Inhibition of sEH by substituted chalcone oxides occurs through electronic stabilization of the covalent enzyme-inhibitor intermediate. These compounds are relatively unstable, particularly in the presence of glutathione [56]. *trans*-3-Phenylglycidol derivatives are enantioselective, slow binding inhibitors of sEH. (2*S*,3*S*)-3-(4-Nitrophenyl)glycidol is the most potent of the 3-phenylglycidol series [55].

Urea derivatives and carbamates are the more recently developed classes of potent, selective and stable sEH inhibitors [57]. The structures of the most commonly used sEH inhibitors are illustrated in (Fig. **2**). *N,N'*-Dicyclohexy-lurea (DCU) is a representative compound of this class (Fig. **2**). The IC_{50} of DCU is 0.16 μM for human sEH [57]. DCU is poorly soluble in H_2O, and this limits its potential

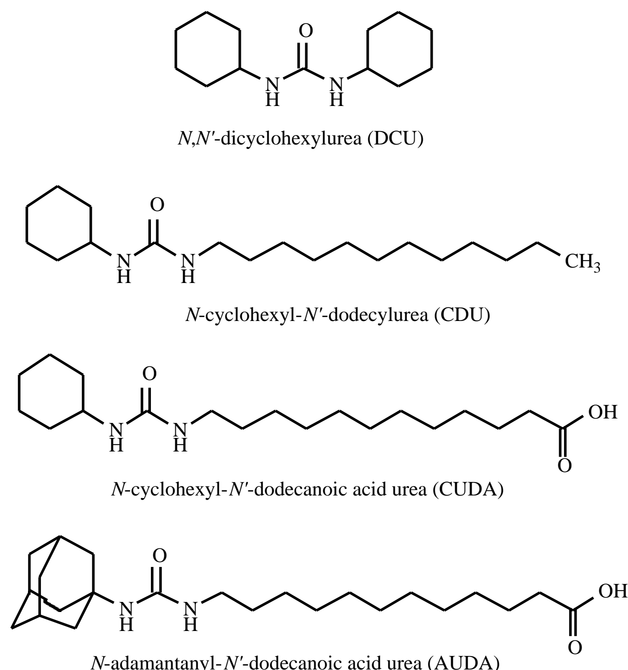

Fig. (2). Structures of representative sEH inhibitors.

usefulness. *N*-cyclohexyl-*N´*-dodecylurea (CDU), in which a hydrocarbon chain replaces the *N´*-cyclohexyl group of DCU, increases the potency of sEH inhibition 8- to 16-fold (IC_{50} of 0.011 μM for human sEH) [58]. *N*-cyclohexyl-*N´*-dodecanoic acid urea (CUDA), which has a carboxylic acid group at the end of the hydrocarbon chain of CDU, increases H_2O solubility without reducing the potency of sEH inhibition. Furthermore, *N*-adamantanyl-*N´*-dodecanoic acid urea (AUDA), in which an adamantanyl group replaces the *N*-cyclohexyl group of CUDA, further increases H_2O solubility without any reduction in potency [58] (Fig. **2**).

A mechanism for the action of the alkylurea inhibitors has been proposed. According to this mechanism, the urea carbonyl oxygen forms hydrogen bonds with the hydroxyl groups of Tyr^{381} and Tyr^{465} of sEH. The urea carbonyl oxygen also interacts with Gln^{382}, which may stabilize the partial negative charge on the epoxide oxygen. The carboxylate side chain of Asp^{333} accepts a hydrogen bond from one of the urea NH-groups in the enzyme-inhibitor complex. Asp^{333} then interacts with the partial positive charge on the urea NH-group. This is similar to the activation of a substrate epoxide ring for nucleophilic attack by Asp^{333} [59].

sEH AND REGULATION OF BLOOD PRESSURE

1. sEH & Hypertension

sEH expression is increased in the pre-hypertensive stage (3 weeks of age) and hypertensive stage (9 weeks of age) in spontaneous hypertensive rats (SHR) as compared with non-hypertensive Wistar-Kyoto rats (WKY) [60]. Expression of sEH in renal cortical tissue and renal microvessels is also increased in rats made hypertensive by the infusion of angiotensin II (Ang II) [61, 62]. Urinary DHETs, the EET hydrolysis products, are increased in SHR and AngII induced hypertension rats [61, 63]. An increased level of urinary DHETs is also found in pregnancy induced hypertensive patients [64]. Furthermore, targeted disruption of the sEH gene in male mice lowers systolic blood pressure [65]. These observations suggest a role for sEH in the regulation of blood pressure.

2. Potential Applications of sEH Inhibitors in Hypertension

EETs have antihypertensive properties and play a part in the maintenance of renal microvascular function. Inhibition of EET hydrolysis by the selective sEH inhibitor DCU reverses the hypertensive phenotype in the SHR rats [63], presumably by maintaining a high level of EET in renal tissue. Administration of CDU (3 mg/d) by intraperitoneal injection for 4 days lowers systolic blood pressure by 30 mm Hg in Ang II hypertensive animals [61]. CDU treatment significantly attenuates the afferent arteriolar diameter responses to angiotensin in hypertensive kidneys from 51% +/- 8% to 28% +/- 7%. Urinary albumin excretion, an index of renal damage, is also lower in CDU-treated hypertensive rats [63]. AUDA also decreases blood pressure and increases the excretion of urinary salt and water in mice with Ang II-induced hypertension [66]. sEH and EETs might also be involved in the pathogenesis of the deoxycorticosterone acetate (DOCA)-salt model of hypertension [67]. Adamantly-dodecyl urea (ADU) lowers the systolic blood pressure and decreases left ventricular weight relative to body weight in DOCA-salt treated rats as compared to DOCA treated rats [67].

Brain sEH seems to play a different role in blood pressure control as compared with peripheral sEH. Although

sEH expression in the hypothalamus and brain stem, two cardioregulatory brain areas, is increased in the SHR rat compared to the WKY rat, inhibition of sEH by intracerebroventricular delivery of AUDA further increases both blood pressure and heart rate in the SHR. AUDA also produces a decrease in the spontaneous baroreceptor reflex gain. The hypertensive effect of sEH inhibition is thought to be a result of an increase in EET-mediated generation of ROS. Therefore, an increased central expression of sEH is a response that may protect against hypertension [68].

The possible mechanism through which a sEH inhibitor lowers blood pressure probably involves the following factors: 1) EETs, but not DHETs, produce vasodilation in the renal circulation [34, 69]. The enhanced renal microvascular reactivity to Ang II that occurs in hypertension is decreased by 11,12-EET [70]. Administration of a sEH inhibitor decreases the hydrolysis of EETs and, therefore, maintains a higher endogenous EET level. This reduces renal vasoconstriction and thereby decreases renin-Ang II-aldosterone-dependent hypertension. 2) sEH inhibition increases the incorporation of EETs into phospholipids, leading to greater EET mobilization when the cell is stimulated. The increased release of EET potentiates the vasorelaxant response [20, 71, 72]. 3) When sEH is inhibited, increased amounts of EET are converted to chain-shortened epoxy fatty acids through -oxidation. Epoxy-16:2, the main chain-shortened epoxy fatty acid that accumulates in the medium under these conditions [20], causes potent vasodilation [73]. 4) sEH inhibition by AUDA increases the excretion of urinary salt and water, responses that are opposite to AngII- aldosterone induced kidney responses. AUDA also decreases the heart rate, and increases the perfusion of the kidney [66]. Figure **3** illustrates the main mechanisms that contribute to the regulation of blood pressure by sEH inhibitors.

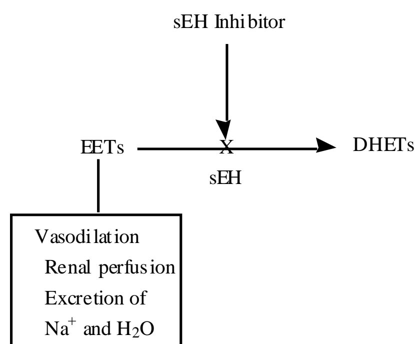

Fig. (3). Main mechanisms of sEH inhibitors as potential antihypertensive drugs.

CURRENT & FUTURE DEVELOPMENTS

In addition to potential applications in the treatment of hypertension, sEH inhibitors have beneficial effects in atherosclerosis [74], acute respiratory distress syndrome [75], and tobacco smoke-induced lung inflammation [76]. However, the effectiveness, safety, pharmacokinetics, and possible drug interactions of sEH inhibitors need to be further investigated, particularly in human subjects. sEH inhibitors that contain carboxyl group such as CUDA and AUDA also activate the peroxisome proliferator activated receptor [77], suggesting that effects in addition to those on EETs metabolism might also play an important role in sEH inhibitor-mediated biological activities.

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