Linking an insect enzyme to hypertension: angiotensin II–epoxide hydrolase interactions

Ding Ai¹,², John Y-J. Shyy³ and Yi Zhu¹,²

¹Cardiovascular Research Center, Shantou University Medical College, Shantou, Guangdong, China; ²Department of Physiology and Pathophysiology, Peking University Health Sciences Center, Beijing, China and ³Division of Biomedical Sciences, University of California, Riverside, California, USA

Derived from arachidonic acid, epoxyeicosatrienoic acids function as antihypertensive and antihypertrophic mediators in the cardiovascular system. Epoxyeicosatrienoic acids are generated by soluble epoxide hydrolase, an enzyme hydrolyzing the epoxide moiety of juvenile hormones in insects, and are endothelium-derived hyperpolarizing factors that induce vessel dilation for cardioprotection. Pharmacological inhibition and genetic ablation of soluble epoxide hydrolase increases the level of epoxyeicosatrienoic acids. Recent findings suggest that the level of soluble epoxide hydrolase in the heart and endothelium is upregulated by angiotensin II in vitro in cultured cardiomyocytes and vascular endothelial cells and in vivo in rodent models. Treatment with soluble epoxide hydrolase-selective inhibitors in angiotensin II–infused hypertensive rats increases the level of epoxyeicosatrienoic acids, with attendant decrease in systolic blood pressure. Shear stress, the physiological stimulation of vessel dilation, downregulates soluble epoxide hydrolase and hence increases epoxyeicosatrienoic acid level in endothelial cells. Because of the close association of the angiotensin II/soluble epoxide hydrolase/epoxyeicosatrienoic acid system and blood pressure regulation, pharmacological inhibition of soluble epoxide hydrolase would be a useful approach to prevent and treat angiotensin II–induced cardiac hypertrophy and hypertension, as well as vascular impairments.

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Epoxide hydrolase (EH) was originally identified as an enzyme hydrolyzing the epoxide moiety of juvenile hormones in all insects. EH orthologs, including soluble epoxide hydrolase (sEH), microsomal EH, cholesterol EH, hepxilin hydrolase and leukotriene A4 hydrolase, are found in mammalian cells. Among them, sEH is composed of two 62-kDa monomeric subunits and mainly localized in the cytosol. The C-terminal region of mammalian sEH contains the catalytic domain that hydrolyzes epoxyeicosatrienoic acids (EETs) to result in the corresponding diols, dihydroxyeicosatrienoic acids.¹ The catalysis of sEH involves the formation and hydrolysis of a covalent acylor alkyl-enzyme intermediate. sEH is distributed in various tissues, including the liver, kidney, lungs, heart, brain, spleen, vascular endothelium and smooth muscle, skin, and leukocytes.¹

EETs, derived from arachidonic acid, are recognized as major regulators of renal and cardiovascular function. Although arachidonic acid is catabolized by three pathways (that is, cytochrome P450 epoxgenases (CYP), cyclooxygenases, and lipoxygenases), CYP2C and CYP2J are the two major human CYP isoforms that convert arachidonic acid to 5,6-, 8,9-, 11,12-, or 14,15-EET (Figure 1). CYP2C and CYP2J can produce all four EET regioisomers, but 11,12- and 14,15-EETs are the predominant products.¹ In mammals, EETs are considered endothelium-derived hyperpolarizing factors that lead to vasodilation in a number of vascular beds. The involved mechanism is EETs activating the large-conductance Ca²⁺-activated K⁺ channels in vascular smooth muscle cells and thus rendering them hyperpolarized.² Independent of their membrane-hyperpolarizing effects, EETs exert anti-inflammatory effects in vascular endothelial cells (ECs) by a mechanism involving inhibition of the transcription factor NF-κB.³ EETs also show potent effects on renal vascular reactivity and tubular sodium and water transport.

The enzyme sEH, through hydrolyzing EETs, has a similar effect on cardiac hypertrophy as that by angiotensin II (Ang II).³ Because of the vasodilating effect of EETs, sEH may also mediate the vasoconstriction effect induced by Ang II. This review summarizes recent findings on the association of sEH and Ang II in the cardiovascular system.
ANG II, BLOOD PRESSURE, AND sEH INHIBITION

Acting at distinct anatomic sites, Ang II is the main effector peptide in the renin-angiotensin-aldosterone system that maintains systemic blood pressure. Ang II also has a detrimental role in cardiovascular impairments such as hypertension, cardiac hypertrophy, and atherosclerosis. Several recent studies of rodent models demonstrated a positive association of sEH, Ang II, and elevated blood pressure. Yu et al. demonstrated that EET hydrolysis greatly increased the renal cortical fractions of spontaneously hypertensive rats (SHRs) compared with normotensive Wistar-Kyoto rats. This augmented EET hydrolysis was consistent with increased expression of sEH in SHR renal microsomes and cytosol. The administration of 1,3-dicyclohexyl urea, the first generation of competitive sEH inhibitors, significantly lowered blood pressure in these animals. Imig’s group showed increased sEH protein expression in the kidney and renal microvessels of rats treated with Ang II. Treating these animals with another sEH inhibitor, 1-cyclohexyl-3-dodecyl urea (CDU, also known as NCND), decreased blood pressure and urinary albumin excretion and reduced the diameter response to Ang II of the afferent arteriole in the hypertensive rats. Fleming’s group showed that pre-treating animals with a water-soluble sEH inhibitor, 12-(3-adamantan-1-yluredio) dodecanoic acid (AUDA), largely attenuated the hypertensive effect of Ang II. Convincingly, the cessation of AUDA treatment increased the blood pressure, which reached the level of the AUDA-untreated hypertensive animals; when AUDA treatment was started again after induction of Ang II-induced hypertension, blood pressure dropped almost to the basal level. The sEH inhibitor could also lower blood pressure and ameliorate renal damage in Ang II-infused and high salt-fed rats. Collectively, these studies suggest that an elevated level of Ang II is associated with increased expression of sEH in blood pressure-regulating tissues and increased systemic blood pressure. Inhibitors of sEH could be used as therapeutic agents for Ang II- and salt-dependent hypertension. The exact mechanism by which various sEH inhibitors exert antihypertensive effects is unclear. Blood pressure lowering may be the result of EETs’ initial reduction of cardiac rates, followed by pressure diuresis resulting from increased perfusion of the kidney. However, a reduction in heart rate was not observed in rats when sEH inhibitors were administered chronically. Alternatively, sEH inhibitors may enhance the vasodilatation effect of EETs on resistant arteries. The addition of EETs or overexpression of P450 epoxygenases in vivo and in vitro resulted in increased expression and activation of eNOS, which suggests that EETs can influence the vascular NO level. Recently, Hercule et al. used N-adamantyl-N′-dodecylurea to show that EETs/dihydroxyeicosatrienoic acids induce endothelial NO release to modulate vascular tone in isolated mesenteric arteries. In that study, the eicosanoids seemed to work by their ability to stimulate NO release.

In addition to regulating blood pressure, sEH may be involved in inflammation. Recent study showed that Ang II- and high-salt diet-induced hypertension in diabetic Goto–Kakizaki rats increased urinary albumin excretion and glomerular and tubular damage. Although the administration of AUDA did not alter blood glucose and blood pressure in the hypertensive Goto–Kakizaki rats, glomerular and tubular damage were decreased. In addition, AUDA treatment attenuated macrophage infiltration and urinary excretion of albumin and monocyte chemoattractant protein-1, which might be due in part to the decreased expression of monocyte chemoattractant protein-1 in the kidney cortex.

THE REGULATION OF sEH BY ANG II IN ECs

Given that EETs have a beneficial effect on vasculature and that sEH can hydrolyze EETs to attenuate vasodilation and anti-inflammatory effects, Ang II may regulate sEH expression and/or activity in the vasculature. Expression of sEH was found to be increased in the renal microvessels of Ang II-induced hypertensive rats. In addition, we showed an increased protein level of sEH in aortic specimens collected from saline-fed SHRs and Ang II-infused Wistar-Kyoto rats. Angiotensin type 1 receptor (AT1) blocker (that is,
Losartan abrogated the increased sEH protein level induced by Ang II, which suggests that the effect was mediated through the AT1 receptor. It is interesting to note that the sEH level was increased in the aortic intima, which is composed of endothelium, but not in the media, which contains mainly vascular smooth muscle cells. In cultured ECs, the expression of sEH at both the protein and mRNA levels was greatly increased by Ang II in a dose- and time-dependent manner. A promoter transfection study further confirmed that the regulation of sEH by Ang II is at the level of transcription.

As a potent mitogen, Ang II binds to its receptor, AT1, to activate several signaling pathways, with consequent upregulation of downstream transcriptional factors, including NF-κB, activator protein 1 (AP-1), nuclear factor of activated T-cell and signal transducers and activators of transcription, which govern the targeting genes in Figure 2. Among them, sEH is a novel targeting gene regulated by Ang II. Sequence analysis revealed one NF-κB-binding site and three AP-1 putative binding sites in the cloned 1.1-kb human sEH promoter. Although Ang II activates both NF-κB and AP-1, the AP-1 site at −446 of the sEH promoter seems to be responsible for the induction of sEH by Ang II. Furthermore, overexpressing c-Jun and its dominant-negative mutant confirmed that c-Jun is necessary and sufficient for sEH induction by Ang II. Recently, Monti et al. identified sEH as a gene related to heart failure susceptibility in rats with spontaneously hypertensive heart failure. Upstream sequencing analysis revealed a 2-nt deletion in the putative sEH promoter in rats with spontaneously hypertensive heart failure as compared with SHRs and Wistar-Kyoto rats. Interestingly, the 2-nt promoter deletion creates an AP-1-binding site in rats with spontaneously hypertensive heart failure. This AP-1-binding site may contribute to increased transcriptional activation, protein expression, and enzyme activity, leading to more rapid hydrolysis of cardioprotective EETs and further suggests the importance of AP-1 in sEH regulation. Thus, binding of Ang II to the AT1 receptor may activate a series of signaling cascades, which in turn, may activate AP-1 to bind to the cognate cis-element at the promoter of the sEH gene. The increased sEH level therefore enhances the hydrolysis of EETs to become dihydroxyeicosatrienoic acids. With decreased level of EETs released from ECs, the paracrine effect of EETs on VSMC hyperpolarization is attenuated, which then increases blood pressure (depicted in Figure 3).

Figure 2 Overview of the signal transduction pathways stimulated by angiotensin II (Ang II) in the cardiovascular system. Ang II binds to angiotensin type 1 receptor (AT1) to activate several signaling pathways, with consequent upregulation of the downstream transcriptional factors, including nuclear factor (NF)-κB, activating protein 1 (AP-1), nuclear factor of activated T-cell (NFAT), and signal transducers and activators of transcription (STATs). Each transcriptional factor governs its targeting genes as listed. sEH is transcriptionally upregulated by the AP-1 pathway. All these genes contribute to the effects of Ang II, including vasoconstriction, vascular inflammation, cell proliferation, myocardial hypertrophy, fibrosis, and renal damage. Because sEH inhibitors can ameliorate most effects induced by Ang II, sEH has an important role in the function of Ang II in the cardiovascular system. sEH, soluble epoxide hydrolase.
Epoxyeicosatrienoic acids; sEH, soluble epoxide hydrolase.

Activating protein 1; AT1, angiotensin type 1 receptor; EETs, which increases blood pressure. Ang II, angiotensin II; AP-1, on vascular smooth muscle cell hyperpolarization is attenuated, EETs released from endothelial cells, the paracrine effect of EETs EETs into dihydroxyeicosatrienoic acids (DHETs). With decreased of sEH cis AP-1 and increases the binding of AP-1 to the cognate ligand-binding domain of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) with kilodaltons in the micromolar range. In the presence of AUDA, EETs increased PPARγ transcriptional activity in ECs. These findings suggest that EETs are PPARγ ligands, which is in line with a previous report that shear stress increased the transcriptional activity of PPARγ in ECs. Indeed, inclusion of AUDA in the perfusing media enhanced but overexpression of sEH reduced the laminar flow-induced PPARγ activity. Mounting evidence has revealed the anti-inflammatory effect of PPARγ and its cognate ligand thiazolidinedione in the cardiovascular system. Shear stress downregulation of sEH in ECs strongly suggests the involvement of the sEH/EET system in vascular integrity.

sEH inhibitors were found to decrease the formation of atherosclerotic lesions. Descending aortas from apolipoprotein E-null mice fed an atherogenic diet while simultaneously infused with Ang II and an sEH inhibitor showed a 53% reduction in atherosclerotic lesions as compared with control aortas. The reduction in atherosclerosis was inversely associated with ratios of 11,12- and 14,15-EET to DHET, which suggests that the reduction in lesions was associated with the inhibition of sEH.

**ANG II, sEH, AND CARDIAC HYPERTROPHY**

Attenuated sEH level and/or activity, with attendant increase in the level of EETs, appears to be beneficial to the heart. Ablation of the mouse sEH gene protects these animals against pressure overload-induced heart failure and cardiac arrhythmias. In contrast, an augmented level of sEH with consequent decrease in EETs may be pathophysiological. We found sEH level increased in myocardia collected from saline-fed SHRs or Ang II-infused Wistar rats. A newly developed sEH inhibitor, 1-(1-methanesulfonyl-piperidin-4-yl)-3- (4-trifluoromethoxy-phenyl) urea (TUPS, 1709), could significantly attenuate the hypertrophic phenotype induced by Ang II, as seen in the ratio of heart to body weight and ventricular thickness. The impressive effect of TUPS may due to its potent urea pharmacophore, excellent oral availability and pharmacokinetic properties. In a canine model, TUPS showed good absorption, distribution, metabolism, and excretion, with very low IC50. In our study, we focused on the effect of TUPS on the heart; TUPS may also have a protective effect on Ang II-induced renal injury.

Although cardiac hypertrophy can result from physiological exercise, mechanical overload, and/or neurohormonal factors, the mechanisms leading to hypertrophy during pathologic and physiologic states are distinct. The application of AUDA was found to lower blood pressure and heart rate in animals with Ang II-induced hypertension but failed to affect blood pressure in animals with phenylephrine-induced hypertension. Consistently, we found sEH expression upregulated in the Ang II-induced pathologic state but not with norepinephrine treatment or exercise. The protein level of sEH did not significantly change in mice with hypertrophy induced by transverse aortic constriction, but an sEH inhibitor could prevent cardiac enlargement. NF-kB activation was inhibited by an sEH inhibitor in this hypertrophy model, but the transcriptional upregulation of sEH by Ang II seems mainly via AP-1, which contributes to the pathological outcome of Ang II-induced cardiac hypertrophy.

In vitro, Ang II upregulated sEH and hypertrophy markers in neonatal cardiomyocytes isolated from rat and mouse. The expression of these marker genes was elevated with
adenovirus-mediated sEH overexpression but decreased with sEH inhibition or in neonatal cardiomyocytes from sEH−/− mice. At the molecular level, Batchu et al.20 reported that EETs enhanced the recovery of ventricular repolarization following ischemia, possibly through the activation of K+ channels and protein kinase A-dependent signaling.

CONCLUSION AND PERSPECTIVES
The above summary of the literature suggests that an above physiological level of Ang II is associated with elevated bioavailability. The above summary of the literature suggests that an above physiological level of Ang II is associated with elevated EET bioavailability. These data support the concept that EETs are physiological regulators of vascular tone. The detailed molecular signaling and the co-activators and co-repressors involved deserve future study. Because EETs are cardiovascular protective, the pathological consequences of the induction of sEH by Ang II would be a decreased level of EETs. Given the four regioisomers of EETs, the specificity of each serving as the substrate of sEH and the mechanisms by which they protect ECs and cardiomyocytes are other possible research topics. In addition, eNOS-derived NO bioavailability is pivotal for the physiological functions of the cardiovascular system; the interaction of Ang II, sEH, and EET merges with the eNOS/NO pathway to modulate vasomotor tone and blood pressure. More information is needed on this interaction and its implications during cardiovascular disease.

The potency of the Ang II/sEH system in modulating blood pressure and the associated cardiovascular impairments suggest the possibility of sEH inhibitors used as pharmacological therapy for hypertension and other Ang II-related cardiovascular diseases. From knowledge of the mechanism of sEH catalysis, transition state inhibitors such as 1,3-dicyclohexyl urea and ACU have been developed. Although potent, these inhibitors are difficult to formulate for delivery.18 Newer versions of sEH inhibitors with improved solubility, including AUDA and TUPS, have been used in animal experiments and show clinical efficacy. Actually, results for the first orally administered sEH inhibitor, AR9281, investigated in a phase I clinical trial in healthy volunteers, has recently been reported (http://www.aretetherapeutics.com/news/2008/121808.html). Future research could investigate the clinical applications of various sEH inhibitors in hypertension, metabolic syndrome, and several inflammatory disorders.

DISCLOSURE
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