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Epoxyeicosatrienoic Acids and 20-Hydroxyeicosatetraenoic Acid on Endothelial and Vascular Function

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Abstract

Endothelial and vascular smooth cells generate cytochrome P450 (CYP) arachidonic acid metabolites that can impact endothelial cell function and vascular homeostasis. The objective of this review is to focus on the physiology and pharmacology of endothelial CYP metabolites. The CYP pathway produces two types of eicosanoid products: epoxyeicosatrienoic acids (EETs), formed by CYP epoxygenases, and hydroxyeicosatetraenoic acids (HETEs), formed by CYP hydroxylases. Advances in CYP enzymes, EETs, and 20-HETE by pharmacological and genetic means have led to a more complete understanding of how these eicosanoids impact on endothelial cell function. Endothelial-derived EETs were initially described as endothelial-derived hyperpolarizing factors. It is now well recognized that EETs importantly contribute to numerous endothelial cell functions. On the other hand, 20-HETE is the predominant CYP hydroxylase synthesized by vascular smooth muscle cells. Like EETs, 20-HETE acts on endothelial cells and impacts importantly on endothelial and vascular function. An important aspect for EETs and 20-HETE endothelial actions is their interactions with hormonal and paracrine factors. These include interactions with the renin-angiotensin system, adrenergic system, purinergic system, and endothelin. Alterations in CYP enzymes, 20-HETE, or EETs contribute to endothelial dysfunction and cardiovascular diseases such as ischemic injury, hypertension, and atherosclerosis. Recent advances have led to the development of potential therapeutics that target CYP enzymes, 20-HETE, or EETs. Thus, future investigation is required to obtain a more complete understanding of how CYP enzymes, 20-HETE, and EETs regulate endothelial cell function.

1. INTRODUCTION

Endothelial cells are recognized as important modulators of vascular function and critical for maintaining hemodynamic homeostasis. The endothelium interfaces with plasma and blood cells to respond to physical forces, blood cells, and endocrine and paracrine circulating factors. The endothelial cell can activate cell signaling pathways and release of autocrine and paracrine factors in response. These endothelial factors can regulate vascular inflammation, platelet aggregation, vascular permeability, vascular smooth muscle cell function and blood flow, and angiogenesis.

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CONFLICT OF INTEREST STATEMENT

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It is well recognized that endothelial cells release three primary paracrine factors in response to shear stress and hormones to regulate vascular smooth muscle cell function. These endothelial-derived relaxing factors include nitric oxide synthase (NOS) generation of nitric oxide (NO), cyclooxygenase (COX) prostacyclin (PGI₂) generation, and cytochrome P450 (CYP) generation of epoxyeicosatrienoic acids (EETs) (Campbell & Fleming, 2010; Furchgott & Vanhoutte, 1989). Although NO is a major vasodilator factor in large arteries, EETs and NO have similar contributions with a lesser contribution from PGI₂ to endothelial-derived relaxation of smaller resistance arteries and arterioles (Campbell & Fleming, 2010; Imig, 2012). Over the past decades these endothelial-derived factors have been demonstrated to have a number of other activities that maintain vascular homeostasis (Fleming, 2001; Imig, 2012).

The number of endothelial-derived cell signaling pathways, endothelial-derived factors, and endothelial cell physiological roles has been greatly expanding. Endothelial cells are a major focus of investigation and pathological roles in cardiovascular diseases are evaluated for potential therapeutic intervention. A significant role for the endothelium is now recognized for immune diseases, diabetes, Alzheimer's disease, and cancer (Bellien & Joannides, 2013; Tacconelli & Patrignani, 2014). Pharmacological manipulation of endothelial NO and COX metabolites has been extensively evaluated and demonstrated promise in many of these diseases (Bellien & Joannides, 2013; Tacconelli & Patrignani, 2014). An emerging area for pharmacological therapeutics is the endothelial-derived CYP metabolites. This review will focus on the physiology and pharmacology of endothelial CYP metabolites.

2. GENERATION AND PRODUCTION OF EETs AND 20-HETE

Endothelial cells produce a large number of hormonal, paracrine, and autocrine factors to regulate cardiovascular function. Identification of CYP-derived EETs as endothelial-derived hyperpolarizing factors (EDHFs) resulted in concentrated efforts to evaluate their contribution to vascular function (Campbell, Gebremedhin, Pratt, & Harder, 1996; Fisslthaler et al., 1999). Another CYP-derived metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), was initially thought to be generated by and act on vascular smooth muscle cells and contribute to the myogenic response and blood flow autoregulatory responses (Imig, Zou, Ortiz de Montellano, Sui, & Roman, 1994; Zou et al., 1996; Zou, Imig, Ortiz de Montellano, Sui, & Roman, 1994). More recently, a contribution of vascular smooth muscle cell-derived 20-HETE to endothelial cell function has emerged (Hoopes, Garcia, Edin, Schwartzman, & Zeldin, 2015). Thus, the generation and regulation of EETs and 20-HETE can greatly contribute to endothelial and cardiovascular function.

EETs and 20-HETE are generated from arachidonic acid by distinct enzymatic CYP pathways (Fig. 1). In general, endothelial CYP2C and CYP2J enzymes generate EETs that can be hydrolyzed to dihydroxyeicosatrienoic acids (DHETs) by the enzyme soluble epoxide hydrolase (sEH; Capdevila & Falck, 2001; Imig, 2013). Endothelial CYP2C and CYP2J epoxygenase enzymes vary depending on the species and organ (Capdevila & Falck, 2001). In addition, CYP epoxygenase enzymes generate different portions of the regioisomeric EETs (5,6-EET; 8,9-EET; 11,12-EET; 14,15-EET) (Capdevila & Falck, 2001; Karara et al., 1993). Likewise, the arachidonic acid metabolite, 20-HETE, is generated by different CYP

enzymes of the CYP4A and CYP4F enzymatic families (Roman, 2002). CYP4A and CYP4F enzymes also vary depending on sex, species, and organ (Capdevila & Falck, 2001). There are also potential interactions between endothelial cell EET- and 20-HETE-generating enzymes (Imig, 2013). This complex regulation of EETs and 20-HETE requires understanding if their pharmacological manipulation is to be used as a therapeutic.

The generation of EETs by CYP2C and CYP2J enzymes has been extensively evaluated in a number of species and organ systems (Capdevila & Falck, 2001). These enzymes are localized to the endoplasmic reticulum and convert arachidonic acid that is released from phospholipid membranes to EETs (Spector, Fang, Snyder, & Weintraub, 2004). CYP epoxygenases add an epoxide group across one of four double bonds to form 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (Spector et al., 2004). EETs are generated as *cis*-epoxides with a high degree of enantiofacial selectivity of (*R,S*) and (*S,R*) enantiomers (Capdevila & Falck, 2001; Spector et al., 2004). CYP2C enzymatic proteins are responsible for the majority of endothelial cell EET generation (Fleming, 2007). Species variation exists among the CYP2C isoforms; however, a significant degree of homology and catalytic activity exists between mouse (CYP2c40 and CYP2c44), rat (CYP2C11 and CYP2C23), and human (CYP2C8) epoxygenase enzymes (Capdevila & Falck, 2001). These CYP2C isoforms generate all four regioisomeric EETs with 11,12-EET and 14,15-EET being at least 70–80% of the total EETs formed (Capdevila & Falck, 2001; Zeldin, 2001). The CYP2J family also is capable of converting arachidonic acid to EETs (Zeldin, 2001). Although CYP2J enzymes preferentially generate 14,15-EET, there are species differences between mouse (Cyp2j5), rat (CYP2J3), and human (CYP2J2) enzymes. CYP2C and CYP2J epoxygenase enzymes have been found in the vasculature and endothelial cells of mice, rats, and humans (Zeldin, 2001). Nevertheless, there is still limited information on the contribution of these enzymes to endothelial and vascular smooth muscle cell function.

An important regulator of EET vascular function is the sEH enzyme. The sEH enzyme carries out catabolic conversions of EETs to their corresponding DHETs (Imig & Hammock, 2009; Morisseau & Hammock, 2013). Interestingly, 14,15-EET, the major regioisomeric EET formed by CYP2C and CYP2J isoforms, is the preferred substrate for sEH (Morisseau & Hammock, 2013). Other metabolic pathways become prominent when sEH activity is low or inhibited (Spector et al., 2004). Chain elongation to form 22-carbon products or β -oxidation to generate 16-carbon epoxy fatty acids are the primary alternative 11,12-EET and 14,15-EET metabolic pathways (Spector et al., 2004). EETs can also be regulated by localization to cell membranes and binding to proteins (Spector et al., 2004). Although EETs in phospholipids represent a very small fraction, EETs could impact function through actions at localized domains (Ellinsworth, Earley, Murphy, & Sandow, 2014; Spector et al., 2004). Binding of EETs to fatty acid-binding proteins could traffic EETs to specific intracellular organelles (Spector et al., 2004). Thus, EET metabolic pathways, EET localization, and EET binding can regulate endothelial cell generated 11,12-EET and 14,15-EET and influence their activity.

Arachidonic acid is acted upon by CYP ω -hydroxylases of the CYP4A and CYP4F enzymatic families to generate 20-HETE (Roman, 2002). CYP ω -hydroxylase enzymes insert a hydroxyl group at the terminal carbon of arachidonic acid. There are several CYP4A

and CYP4F isoforms that vary among mice (Cyp4a10 and Cyp4a12), rats (CYP4A1, CYP4A2, CYP4A3, CYP4A8, and CYP4F1), and humans (CYP4A11, CYP4A22, and CYP4F2; Capdevila & Falck, 2001; Roman, 2002). CYP4F2 is the predominant 20-HETE synthase in humans followed by CYP4A11 (Roman, 2002). The CYP4F expression varies between species and has been found in kidney, liver, and vascular smooth muscle cells (Roman, 2002). Likewise, CYP4A enzymes have been localized to the kidney and renal vasculature (Imig et al., 1994; Zou et al., 1994). Endothelial progenitor cells (EPCs) have been demonstrated to produce 20-HETE but vascular endothelial cells are devoid of 20-HETE synthesis capacity (Wu & Schwartzman, 2011). Even though vascular synthesis of 20-HETE occurs primarily in the vascular smooth muscle cell, myeloid cells in the peripheral blood, neutrophils, platelets, and bone marrow cells produce 20-HETE that could impact on endothelial cell function (Hoopes et al., 2015; Wu & Schwartzman, 2011).

CYP4A enzymes are regulated by hormones and can metabolize other substrates, while 20-HETE can be further metabolized. A key feature of CYP4A enzymes is their sex-dependent expression and regulation by androgen (Holla et al., 2001; Wu et al., 2013). Cyp4a12 is expressed in male mice kidneys but is near undetectable levels in the female kidney (Holla et al., 2001). In addition, studies have demonstrated that the murine Cyp4a12 is androgen regulated and this regulation can increase vascular resistance (Holla et al., 2001; Wu et al., 2013). Androgen is a potent inducer of 20-HETE synthesis and has been implicated in increased vascular resistance and development of hypertension (Holla et al., 2001; Wu & Schwartzman, 2011). CYP4A enzymes can metabolize other fatty acids including laurate and ω -hydroxylate EETs (Capdevila & Falck, 2001). CYP4A and CYP4F enzymes also generate other carbons 16–19 on arachidonic acid to produce subterminal HETEs (Capdevila & Falck, 2001). Like EETs, 20-HETE can be metabolized by β -oxidation to generate shorter chain length products (Roman, 2002). In addition, COX enzymes metabolize 20-HETE to produce vasoconstrictor endoperoxides or vasodilator prostanoids (Carroll, Garcia, Falck, & McGiff, 1992). Thus, these other CYP4A and CYP4F metabolites and 20-HETE metabolites have been demonstrated to have vascular actions.

This review will focus on CYP metabolites of arachidonic acid that are the most abundant and well-characterized metabolites. An emerging area for CYP enzymes is the potential to act on ω -3 polyunsaturated fatty acids, eicosapentaenoic acid and docosahexanoic acid, to generate metabolites that have endothelial cell actions (Ulu et al., 2014; Westphal, Konkel, & Schunck, 2011). Epoxyeicosatrienoylglycerols are a novel class of arachidonic acid metabolites that have been described and demonstrated to cause vasodilation through the activation of cannabinoid CB1 receptors (Chen et al., 2008). Undoubtedly, these and other novel CYP epoxygenase and hydroxylase metabolites will be the focus of future investigations.

3. PHARMACOLOGICAL AND GENETIC TOOLS

A major driving force responsible for our understanding of the contribution of EETs and 20-HETE to endothelial function and vascular homeostasis is the development of pharmacological and genetic tools. CYP enzymatic inhibitors have evolved from nonselective CYP inhibitors to selective epoxygenase and hydroxylase inhibitors (Imig,

2013). Inhibitors of sEH have also been utilized to prevent the conversion of EETs to DHETs (Imig, 2013). 20-HETE and EET structure-activity relationship studies have led to the development of selective agonists and antagonists (Gauthier, Falck, Reddy, & Campbell, 2004). Many of these pharmacological agents can now be used in animals. Genetic manipulation has also been done at the cellular and whole animal levels. CYP2C, CYP2J, CYP4A, CYP4F, and sEH enzymes can be overexpressed or deleted in cellular systems, rats, and mice at the whole animal levels or in an endothelial and vascular smooth muscle cell-specific manner.

CYP enzymatic inhibitors were the first major breakthrough that allowed for evaluation of endothelial and vascular function. Initially, general CYP inhibitors like 17-ODYA were utilized and compared with phospholipase A₂ (PLA₂), COX, and lipoxygenase (LOX) inhibitors (Imig, 2013). Unfortunately, 17-ODYA could not discriminate between epoxygenase and hydroxylase pathways. Antifungal agents such as miconazole were initially used as more selective epoxygenase inhibitors; however, conclusions made with studies employing these agents were not clear-cut. More selective inhibitors were made possible because great strides were made in understanding the biochemistry of epoxygenase and hydroxylase enzymes.

Selective CYP epoxygenase and hydroxylase inhibitors developed included small-molecule mechanism-based inhibitors (Wang et al., 1998). The most widely utilized small molecules act as competitive inhibitors (Wang et al., 1998). An oleinic compound, DDMS, exhibits a high degree of selectivity for inhibiting ω -hydroxylation of arachidonic acid with no selectivity for CYP4A isoforms (Wang et al., 1998). Another widely used and characterized CYP hydroxylase inhibitor is HET0016. HET0016 administered chronically to rats can selectively inhibit 20-HETE formation (Roman, 2002). Likewise, epoxygenase inhibitors PPOH and MS-PPOH selectively inhibit microsomal epoxygenase with little effect on hydroxylase activity at high micromolar concentrations (Wang et al., 1998). Other enzymatic inhibitors were developed to prevent the conversion of EETs to DHETs by sEH (Imig & Hammock, 2009). Potent transition state sEH inhibitors were developed based on the knowledge of the catalytic mechanism of the enzyme (Imig & Hammock, 2009). The most widely used sEH inhibitor is AUDA that can be administered to rodents chronically (Imig & Hammock, 2009). Development of sEH inhibitors for use in humans has advanced and clinical trials in the areas of hypertension, diabetes, and chronic obstructive pulmonary disease conducted (Bellien & Joannides, 2013; Marino, 2009). Investigators have utilized these enzymatic inhibitors to determine the contribution of CYP epoxygenase, CYP hydroxylase, and sEH on endothelial and vascular function.

A key to our understanding of the endothelial actions for EETs was a full understanding to the structure-activity relationship for EETs. EET regioisomer-specific agonists were synthesized to improve solubility and resist autooxidation and metabolism by sEH (Gauthier et al., 2004; Imig, 2012). Initial steps were the replacement of the carboxylic acid with a sulfonamide to resist β -oxidation (Imig, 2012). A structure-activity relationship for EETs emerged as additional modifications were made to the various EET regioisomers. It became evident that the endothelial and biological actions for 11,12-EET and 14,15-EET had three main components. 11,12-EET and 14,15-EET required an acidic carboxyl group, [8] olefin

bond, and a *cis*-epoxide (Imig, 2012). EET mimetics/analogues have now been developed to point where they can be used in endothelial cell systems, isolated vascular studies, and administered orally to rodents (Imig, 2012). Additional information from EET structure relationship studies and endothelial cell signaling studies has led to the development of EET antagonists.

Development of EET antagonists has been another essential pharmacological tool to determine EET endothelial actions. EET antagonists are based on the general EET structure with 14,15-EEZE being the most widely utilized EET antagonist (Gauthier et al., 2004). A key component to 14,15-EEZE is that it lacks an 8,9- and 11,12-olefin bond (Gauthier et al., 2004). 14,15-EEZE is a general nonselective EET antagonist that cannot discern the actions of the various regioisomeric EETs (Gauthier et al., 2002). More recently, EET antagonists that selectively inhibit the vascular actions of 11,12-EET or 14,15-EET have been developed (Bukhari et al., 2012). Experimental studies with these selective 11,12-EET and 14,15-EET antagonists have determined unique contributions for 14,15-EET to flow-mediated dilation in mesenteric resistance arteries (Bukhari et al., 2012). Thus, we now have the pharmacological means to determine the endothelial cell functions for specific EET regioisomers.

20-HETE agonists and antagonists have been developed to better understand the contribution of 20-HETE to vascular function (Roman, 2002). Development of 20-HETE analogs has determined that the presence of a hydroxyl group at C20 or C21 is critical for vascular activity (Alonso-Galicia, Falck, Reddy, & Roman, 1999). In addition, the retention of olefin bonds in the 5,6- and 14,15-carbons is necessary for 20-HETE analogs to retain agonist activity (Alonso-Galicia et al., 1999). These structure-activity relationship findings led to the development of 20-5,14-HEDGE which is the most widely utilized 20-HETE analog. 20-5,14-HEDGE has been successfully administered *in vivo* to rodents; however, β -oxidation and water solubility limit potential for clinical applications (Williams, Murphy, Burke, & Roman, 2010). On the other side of the coin is the development of 20-HETE antagonists (Fernandez, Gonzalez, Williams, Roman, & Nowicki, 2012; Williams et al., 2010). Early studies found that 5-HETE, 15-HETE, and 19-HETE could antagonize the vascular actions of 20-HETE (Alonso-Galicia et al., 1999). This finding led to the development of 20-HEDE that lacks double bonds at the 8,9- and 11,12-positions to resist COX metabolism (Roman, 2002). More recently, a 20-HETE antagonist, SOLA, with greater solubility and oral activity has been used in chronic animal studies (Chen, Ackerman, & Guo, 2012; Gangadhariah et al., 2015). Accordingly, 20-HETE agonists and antagonists have progressed to a state where their cardiovascular therapeutic potential can be evaluated.

Genetic manipulation has provided interesting insight as to the contribution of CYP4A, CYP2C, CYP2J, and sEH enzymes to endothelial function and vascular homeostasis. Approaches have decreased or increased enzymatic protein expression in endothelial cell cultures and endothelial cells at the whole animal level (Capdevila, Falck, & Imig, 2007; Imig, 2012). These genetic manipulations discovered that arachidonic acid metabolism by these enzymes is very complex and identified previously unknown regulation and interactions of these enzymes. It has also greatly enhanced our understanding of EETs and

20-HETE to endothelial cell biology and pathology. More importantly, it has found novel therapeutic targets for endothelial and vascular diseases.

Epoxygenase and sEH enzymes have been genetically manipulated by whole animal gene depletion, and endothelial cell-specific gene deletion or overexpression (Imig, 2012). Endothelial cell culture systems lose expression of epoxygenase enzymes in early passages (Fleming, 2007). This has provided an opportunity to determine the contribution of specific CYP2C and CYP2J enzymes to endothelial cell function. Although epoxygenase enzymes of many species have been evaluated, human CYP2C8 and CYP2J2 expression in endothelial cells has provided excellent insight on their contribution to endothelial cell biology (Imig, 2012). Murine genetic manipulation for Ephx2, CYP2C, and CYP2J enzymes has included global deletion, endothelial cell deletion, and endothelial cell overexpression (Imig, 2012). These genetic-manipulated mice have provided considerable insight on the impact of these enzymes and their metabolites to blood pressure regulation, vascular remodeling, vascular inflammation, and atherosclerosis.

Endothelial cell and vascular 20-HETE biosynthesis can be regulated by genetically manipulating CYP4A and CYP4F expression (Wu & Schwartzman, 2011). CYP4A and CYP4F enzymes have been knocked down or overexpressed in cell culture systems (Roman, 2002; Wu & Schwartzman, 2011). Expression of CYP4A2 in vivo in rat endothelial cells has been accomplished by adenovirus intravenous injection (Wu et al., 2013). Antisense nucleotide CYP4A1/CYP4A2 inhibition in spontaneously hypertensive rats (SHRs) resulted in decreased 20-HETE levels (Sodhi et al., 2010). The CYP4A enzyme has been genetically manipulated in the Dahl salt-sensitive (SS) rat by introgression of CYP4A alleles for the normotensive Brown-Norway rat into the Dahl SS genetic background (Lukaszewicz & Lombard, 2013). This manipulation resulted in a decrease in 20-HETE production in the Dahl SS rat (Lukaszewicz & Lombard, 2013). Cyp4a genes have also been manipulated in mice. Cyp4a14 gene deficiency in mice resulted in increased Cyp4a12(a) expression and increased 20-HETE levels (Holla et al., 2001). On the other hand, Cyp4a10 gene deficiency in mice did not change Cyp4a12(a) expression or 20-HETE levels (Capdevila, Pidkovka, Mei, et al., 2014). Interestingly, Cyp4a10 $-/-$ mice had decreased Cyp2c44 epoxygenase expression (Capdevila et al., 2014). These findings highlight the complex regulation of CYP hydroxylase and epoxygenase enzymes and provide evidence to interactions between these enzymatic pathways. The overall impact of interactions between hydroxylase and epoxygenase pathways on vascular and endothelial study is not completely understood.

4. ENDOTHELIAL AND VASCULAR BLOOD FLOW REGULATION

CYP epoxygenase and hydroxylase have actions on endothelial and vascular smooth muscle cells to influence organ blood flow (Imig, 2012; Roman, 2002; Fig. 2). Initial studies evaluated the effects of EETs, DHETs, and 20-HETE on organ blood flow. Experimental studies in isolated blood vessels were also conducted (Imig, 2012; Roman, 2002). Likewise, experiments were conducted utilizing pharmacological CYP inhibitors (Imig, 2012; Roman, 2002). Two exciting findings on EETs and 20-HETE and blood flow regulation emerged from these early studies. First, identification of EETs as EDHFs peaked interest in CYP epoxygenase metabolites (Campbell et al., 1996; Fisslthaler et al., 1999). Second, 20-HETE

was identified as a critical paracrine factor in blood flow autoregulation (Zou et al., 1994). Numerous investigations have gone on to provide significant detail on EET and 20-HETE endothelial and vascular smooth muscle cell signaling mechanisms. This section will focus on endothelial and vascular regulation by EETs and 20-HETE.

EETs were first described to increase intestinal arteriolar blood flow and subsequent experimental evidence was demonstrated in renal, cerebral, and coronary vasculatures (Imig, 2012; Proctor, Falck, & Capdevila, 1987). EET vasodilation has been demonstrated in rodent and nonrodent species including human bloodvessels (Imig, 2012; Larsen et al., 2006). In general, there is agreement that the most abundant EET regioisomers 11,12-EET and 14,15-EET are vasodilators. There is evidence that 5,6-EET and 8,9-EET cause vasoconstriction that is dependent on metabolism by COX enzymes and activation of thromboxane receptors (Carroll, Balazy, Margiotta, Falck, & McGiff, 1993; Imig, Navar, Roman, Reddy, & Falck, 1996; Imig, Zou, et al., 1996). On the other hand, DHETs that are generated by sEH have been demonstrated to be less active than the corresponding EETs (Imig, Navar, et al., 1996; Imig, Zou, et al., 1996). Additional studies demonstrated that nitric oxide-independent dilation in response to bradykinin or acetylcholine was blocked by CYP inhibition (Imig, Falck, Wei, & Capdevila, 2001; Wang, Borrego-Conde, et al., 2003; Wang, Trotter, & Loutzenhiser, 2003). This led to the idea that EETs could be EDHFs responsible for the nitric oxide- and COX-independent dilator response. Ensuing experimental evidence provided definitive proof that EETs were generated in endothelial cells and acted on the adjacent vascular smooth muscle cells to cause hyperpolarization and vasodilation (Campbell & Fleming, 2010; Pfister, Gauthier, & Campbell, 2010). This seminal discovery led to numerous investigations to further delineate EET regioisomeric vascular actions and cell signaling mechanisms.

Endothelial-derived EETs act on vascular smooth muscle cell K^+ channels resulting in vascular smooth muscle cell hyperpolarization and relaxation (Campbell, Holmes, Falck, Capdevila, & Gauthier, 2006; Li, Zhang, Ge, & Campbell, 2002). This vascular smooth muscle cell response appears to be primarily mediated by the large-conductance calcium-activated K^+ (K_{Ca}) channel (Archer et al., 2003; Dimitropoulou et al., 2007; Li et al., 2002). There is also evidence that the ATP-sensitive K^+ (K_{ATP}) participates in the EET-mediated dilation of mesenteric resistance arteries (Chen & Cheung, 1996; Imig, 2012). 11,12-EET and 14,15-EET cell signaling mechanisms have been the most widely studied and EET analogs for these regioisomers have been essential to these experimental studies (Sudhahar, Shaw, & Imig, 2010). These studies have determined that EET-mediated activation of K_{Ca} channels requires the G protein G_{α_s} and activation of the cAMP-protein kinase A (PKA) cell signaling pathway (Dimitropoulou et al., 2007; Imig, Inscho, Deichmann, Reddy, & Falck, 1999). Additional evidence points to a contribution of TRP channels and localized increases in calcium resulting in a calcium spark to activate K_{Ca} channels (Fleming et al., 2007; Ma et al., 2015; Vriens et al., 2005; Watanabe et al., 2003). Additional evidence suggests that 11,12-EET can act to hyperpolarize the endothelial cell and this leads to vascular smooth muscle cell hyperpolarization via gap junctions and the ouabain-sensitive Na^+-K^+ -ATPase (Ellinsworth et al., 2014; McSherry et al., 2006). EETs also contribute to the conduction of hyperpolarization along an arteriole in response to acetylcholine (McSherry et al., 2006). This finding suggests that EETs can act in a manner other than as an EDHF to cause

vasodilation. Overall, there is significant evidence that EETs can act as an EDHF on vascular smooth muscles and in a non-EDHF manner on endothelial cells to cause vasodilation.

Although initial studies were controversial as to the vascular actions of 20-HETE, this CYP hydroxylase metabolite has now clearly been defined as a vasoconstrictor (Alonso-Galicia et al., 1999; Imig, Navar, et al., 1996; Imig, Zou, et al., 1996; Ma et al., 1993). 20-HETE has been demonstrated to be a potent constrictor of cerebral, renal, and mesenteric resistance arteries (Roman, 2002). Vascular smooth muscle cell signaling actions of 20-HETE are in direct opposition to the EET cell signaling actions. 20-HETE inhibits K_{Ca} channels leading to vascular smooth muscle cell depolarization and increases intracellular calcium (Imig, Navar, et al., 1996; Imig, Zou, et al., 1996; Zou et al., 1996). Direct activation of L-type calcium channels and protein kinase C (PKC) also contributes to 20-HETE-mediated vasoconstriction (Ma et al., 1993; Zou et al., 1996). An interesting interaction between 20-HETE and the endothelial-derived relaxing factor, nitric oxide, could also participate in the regulation of vascular tone (Alonso-Galicia, Drummond, Reddy, Falck, & Roman, 1997). Nitric oxide inhibits 20-HETE formation and this decrease in 20-HETE contributes to a cGMP-independent dilator action of nitric oxide (Alonso-Galicia et al., 1997). These 20-HETE vascular smooth muscle cell signaling events are consistent with the concept that 20-HETE is a key contributor to vascular smooth muscle cell autoregulatory responses.

Even though there has been extensive evidence for EETs as an EDHF and 20-HETE as a critical contributor to blood flow autoregulation, the contribution of these CYP metabolites to overall endothelial and vascular function has greatly expanded. EETs and 20-HETE are now recognized to be critically involved in angiogenesis and vascular inflammation.

5. ANGIOGENESIS AND VASCULAR REMODELING

Angiogenesis is one of the few areas where EET and 20-HETE have similar biological actions (Fig. 3). 20-HETE and EETs have proangiogenic activities that are alike in many respects but uniquely regulated depending on the setting (Imig, 2012; Wu & Schwartzman, 2011). These CYP metabolites have overlapping and distinct cell signaling mechanisms that are utilized to promote angiogenesis. Vascular remodeling is also influenced by 20-HETE and EETs and like most biological activities for these CYP metabolites have opposing ying/yang activities (Hoopes et al., 2015; Imig, 2012). Therefore, in physiological and pathological states the contribution of EETs and 20-HETE to angiogenesis and vascular remodeling is complex and depends on the prevailing metabolites.

There is significant evidence that EETs can contribute to angiogenesis and endothelial cell proliferation (Fleming, 2007; Pozzi et al., 2005; Webler et al., 2008; Zhang, Cao, & Rao, 2006). EETs or overexpression of CYP2C9 to cultured endothelial cells resulted in angiogenesis (Fleming, 2007). EETs or overexpression of various CYP epoxygenase enzymes induces angiogenesis in matrigel plugs in rodents and increases the capillary density following in ischemic limb models (Pozzi et al., 2005). EETs appear to contribute to endothelial cell migration and degradation of extracellular matrix (Munzenmaier & Harder, 2000; Pozzi et al., 2005). Vascular endothelial growth factor (VEGF) stimulated endothelial

cell proliferation and tube formation involves EET generation (Cheranov et al., 2008; Webler et al., 2008; Yang, Wei, Pozzi, & Capdevila, 2009). Likewise, EETs can increase VEGF expression as a positive feedback mechanism to further increase endothelial cell proliferation (Cheranov et al., 2008; Webler et al., 2008). These findings demonstrated that EETs contribute importantly to VEGF angiogenic and endothelial cell proliferative actions.

EETs have endothelial cell signaling actions that promote proliferation. Endothelial cell proliferation is increased by CYP2C overexpression or in response to administration of any of the four regioisomeric EETs (Michaelis et al., 2003; Potente, Michaelis, Fisslthaler, Busse, & Fleming, 2002). Two primary endothelial cell signaling pathways have been implicated in EET-induced proliferation. EET activation of MAPK and PI3K/Akt endothelial signaling are major contributors (Potente, Fisslthaler, Busse, & Fleming, 2003; Wang et al., 2005; Webler et al., 2008). Activation of p38MAPK and inactivation of cJun NH2-terminal kinase (JNK) result from CYP2C9 overexpression in human umbilical vein endothelial cells (HUVECs) (Michaelis et al., 2003). 11,12-EET reduces the cyclin D1 inhibitory protein, P27^{kip1}, and activates PI3K/Akt to inhibit forkhead transcription factors (FOXO; Potente et al., 2003). EET activation of the EGF receptor and release of heparin-binding EGF-like growth factor (HB-EGF) appear to be upstream of these endothelial cell signaling pathways (Fleming, 2007). Lastly, there are interactions between EETs and COX-2 mediated by the endothelial cell cAMP/PKA pathways. 11,12-EET or CYP2C9 overexpression activates endothelial cell cAMP/PKA pathway to stimulate cAMP response element-binding (CREB) protein to stimulate COX-2 expression (Michaelis et al., 2003). Nevertheless, the coordinated endothelial cell signaling activities by which EETs contribute to endothelial cell proliferation and angiogenesis remain to be explored.

Although many of the biological activities of EETs and 20-HETE are opposing, 20-HETE acts similar to EETs to promote angiogenesis (Chen et al., 2012; Hoopes et al., 2015). 20-HETE can increase endothelial cell VEGF expression via MAPK activation (Hoopes et al., 2015). A major contributor to 20-HETE endothelial cell proliferation appears to be stimulation of reactive oxygen species (ROS) and the production of hypoxia-inducible factor-1 α (HIF-1 α ; Chen et al., 2012). CYP4A overexpression or 20-HETE activates angiogenic factors in endothelial cells (Chen et al., 2012). Intriguingly, endothelial cells from mice with endothelial-specific expression of CYP4F2 exhibited increased growth and tube formation that was VEGF dependent (Cheng et al., 2014). There is also evidence that 20-HETE contributes to angiogenesis associated with tumor growth involves PI3K signaling (Wu & Schwartzman, 2011). Thus, 20-HETE-induced endothelial cell proliferation and angiogenesis have similarities and differences when compared to EETs.

A unique aspect for 20-HETE to regulate angiogenesis is actions on EPC function. EPCs derived from HUVECs express CYP4A11, CYP4A22, and CYP4F2 hydroxylase enzymes and generate 20-HETE (Chen et al., 2014). 20-HETE enhances EPC secretion of key proangiogenic factors such as VEGF, HIF-1 α , and stromal-derived factor-1 (SDF-1; Chen et al., 2014). These EPC-secreted factors recruit bone marrow-derived stem cells to promote angiogenesis (Chen et al., 2012). CYP hydroxylase inhibitors or 20-HETE antagonists stop EPC proliferation and migration and EPC-induced endothelial cell differentiation (Chen et

al., 2014). Thus, CYP hydroxylases and 20-HETE regulate EPC function and related angiogenic responses.

Vascular remodeling occurs in many cardiovascular disease states and involves structural reorganization of blood vessels in response to stimuli to induce collagen synthesis and reorganization of the vascular wall extracellular matrix. Matrix metalloproteinases (MMPs) and inflammatory signals are key contributors to vascular remodeling that are influenced by 20-HETE and EETs (Hoopes et al., 2015; Imig, 2012). 20-HETE can promote vascular remodeling, whereas endothelial-derived EETs oppose vascular remodeling (Hoopes et al., 2015; Imig, 2012). Renal arteries can be induced to remodeling by 20-HETE (Garcia et al., 2015; Hoopes et al., 2015). In addition, mice with genetic manipulation to induce Cyp4a12 expression resulting in increased 20-HETE levels result in vascular remodeling (Ding et al., 2013). Vascular remodeling in the Cyp4a12 transgenic mice can be blocked by the 20-HETE antagonist, 20-HEDGE (Ding et al., 2013). On the other hand, vascular remodeling is decreased in Ephx2 $-/-$ mice or by sEH inhibition (Davis et al., 2002; Simpkins et al., 2010). EETs also have actions on to decrease vascular smooth muscle cell migration and proliferation (Fleming, 2007). Vascular hypertrophic remodeling and collagen deposition in the middle cerebral artery of stroke-prone SHR are attenuated by sEH inhibition (Simpkins et al., 2009). Likewise, flow-induced remodeling of the carotid artery in rats and mice was reduced by sEH inhibition or Ephx2 gene deficiency (Simpkins et al., 2010). Femoral artery wire-induced endothelial injury and neointimal formation were not decreased by sEH inhibition or Ephx2 gene deficiency (Simpkins et al., 2010). These findings are consistent with the notion that endothelial-derived EETs are required for sEH inhibition to oppose vascular remodeling. Consequently, endothelial-derived EETs will have opposite actions compared to vascular smooth muscle cell-derived 20-HETE on vascular remodeling.

6. ENDOTHELIAL AND VASCULAR INFLAMMATION

Regulation of vascular inflammation by CYP eicosanoids has been an area of extensive investigation. 20-HETE and EETs can influence vascular smooth muscle cell and endothelial cell inflammatory responses (Hoopes et al., 2015; Imig, 2012). In this regard, EETs are antiinflammatory and 20-HETE is inflammatory (Hoopes et al., 2015; Imig, 2012). These opposing vascular inflammatory actions for EETs and 20-HETE on inflammation are mediated via similar cell signaling pathways.

20-HETE promotes vascular inflammation through endothelial cell activation to increase adhesion molecules and proinflammatory cytokines (Hoopes et al., 2015). In ischemia-reperfusion injury models 20-HETE inhibition decreased vascular inflammation possibly via decreased oxidative stress (Dunn et al., 2008; Hoopes et al., 2015). Decreased 20-HETE synthesis also reduces vascular TNF α , IL-1 β , and IL-6 expression (Hoopes et al., 2015). In the other direction, 20-HETE increases vascular ROS and NF κ B activation (Ishizuka et al., 2008). Treatment of endothelial cells with 20-HETE leads to activation of NADPH oxidases and NF κ B and increased expression of adhesion molecules (Ishizuka et al., 2008). MAPK appears to be involved in the endothelial cell inflammatory response because inhibition of MAPK prevents 20-HETE-induced inflammation (Ishizuka et al., 2008). Taken together, 20-HETE inflammatory actions are dependent on ROS and NF κ B activation.

There is significant evidence that EETs and sEH inhibition have vascular antiinflammatory actions (Deng et al., 2011; Imig, 2012; Node et al., 1999). EETs appear to oppose endothelial cell inflammation via inhibition of NF κ B activation (Deng et al., 2011). Likewise, EETs and EET analogs inhibit vascular smooth muscle cell TNF α -induced VCAM-1 adhesion molecule expression (Khan et al., 2013; Node et al., 1999). Vascular antiinflammatory actions of EETs or sEH inhibition are mediated by inhibition of phosphor-IKK-derived NF κ B activation (Deng et al., 2011; Node et al., 1999). Another mechanism by which EETs are antiinflammatory appears to be through activation of endothelial cell peroxisome proliferator-activated receptor γ (PPAR γ) (Liu, Zhang, Schmelzer, et al., 2005). These findings are in agreement with the well-described interactions between PPARs and EETs. Thus, sEH inhibitors and EET analogs are being tested for their therapeutic potential to treat vascular inflammation and atherosclerosis.

7. ENDOCRINE, PARACRINE, AND AUTOCRINE INTERACTIONS

CYP eicosanoids have been demonstrated to contribute to and regulate endothelial and vascular responses evoked by endocrine, paracrine, and autocrine factors (Imig, 2012; Roman, 2002). Interactions between CYP eicosanoids and the renin-angiotensin system, purinergic system, adenosine, nitric oxide, and endothelin have been extensively evaluated. In general, 20-HETE acts as a key mediator for the endothelial and vascular actions of these endocrine, paracrine, and autocrine factors (Roman, 2002). On the other hand, endothelial-derived EETs act to oppose the actions of these factors (Imig, 2012). Thus, it is well recognized that a balance between EETs and 20-HETE and appropriate responses to endocrine, paracrine, and autocrine factors is required for proper endothelial cell function and vascular homeostasis.

20-HETE and EET interactions with the renin-angiotensin system at the level of the endothelial cell and vasculature are required for endothelial cell function (Fig. 4). Endothelial-derived EETs have been demonstrated to oppose angiotensin II vasoconstrictor actions (Imig & Deichmann, 1997; Kohagura et al., 2000). In the other direction, angiotensin II can increase endothelial cell sEH expression to decrease EETs (Ai et al., 2007). Decreased levels of endothelial cell Ephx2 mRNA expression and sEH protein expression in response to angiotensin II are the result of transcriptional factor AP-1 activation (Ai et al., 2007). Angiotensin-converting enzyme (ACE) inhibition can increase EET levels and this is thought to be due to the increase in bradykinin levels (Matsuda et al., 2004). Evidence also suggests that angiotensin type 2 (AT2) receptors increase endothelial EET levels in response to angiotensin II that acts to blunt the angiotensin II vasoconstrictor response (Imig, 2012; Matsuda et al., 2004). On the other hand, 20-HETE appears to contribute to angiotensin II vasoconstriction (Alonso-Galicia, Maier, Greene, Cowley, & Roman, 2002). Experimental evidence demonstrates that angiotensin II can increase vascular CYP4A expression and 20-HETE levels (Alonso-Galicia et al., 2002; Croft, McGiff, Sanchez-Mendoza, & Carroll, 2000). 20-HETE also contributes to the ability of angiotensin II to cause artery neointimal thickening (Hoopes et al., 2015). More recently it has been recognized that 20-HETE can increase aspects of the reninangiotensin system. Cultured endothelial cells incubated with 20-HETE have a big increase in ACE expression (Cheng et al., 2014). Likewise, lentivirus expression of the CYP4A2 cDNA under the control of an endothelial-specific promoter

resulted in increased aortic ACE and angiotensin type 1 (AT1) receptor expression (Cheng et al., 2014). These findings strongly implicate 20-HETE as a major contributor to angiotensin II-mediated endothelial and vascular actions. Overall, CYP eicosanoid investigations demonstrate the opposing endothelial interactions between 20-HETE, EETs, and the renin-angiotensin system.

There have also been extensive studies on interactions between CYP eicosanoids and the purinergic system. In this case experimental evidence supports the concept that the purinergic factor adenosine interacts with endothelial cell EETs and that 20-HETE interacts with the purinergic factor ATP. 20-HETE interactions with ATP and the purinergic P2X receptors are important for autoregulation of blood flow (Imig, 2013). 20-HETE synthase inhibition or 20-HETE antagonism significantly attenuates arteriolar constriction to ATP or P2X receptor agonists (Zhao, Falck, Gopal, Inscho, & Imig, 2004; Zhao, Inscho, Bondlela, Falck, & Imig, 2001). This interaction appears to be at the level of the vascular smooth muscle cell because 20-HETE inhibition markedly reduces the increase in vascular smooth muscle cell calcium to P2X receptor but not P2Y receptor agonists (Zhao, Falck, et al., 2004). Thus, 20-HETE appears to be a critical cell signaling factor responsible for ATP P2X receptor-mediated autoregulatory responses. Unlike 20-HETE, EETs do not appear to influence arteriolar or vascular smooth muscle calcium responses to P2X receptor activation (Zhao, Falck, et al., 2004). Interestingly, EETs appear to interact with adenosine receptor-mediated vascular responses. EETs contribute to vasodilation in response to adenosine activation of A2A receptors (Carroll et al., 2006; Cheng et al., 2004). This finding is further supported by the fact that epoxxygenase inhibition attenuates vasodilation in response to adenosine or A2A receptor agonists (Cheng et al., 2004). Adenosine through A2A receptor activation and cAMP increases endothelial cell EET generation (Cheng et al., 2004). EETs have also been demonstrated to contribute to adenosine A2B receptor cerebral arteriolar dilation (Alkayed et al., 1997). Taken together, there is clear evidence for 20-HETE interactions with ATP and P2X receptor activation and EET interactions with adenosine and A2A and A2B receptor activation that impact on vasoconstrictor and vasodilator responses.

Major interactions between endothelial factors endothelin-1 and nitric oxide and 20-HETE importantly influence vascular function. Endothelin-1 increases 20-HETE generation by arterioles (Imig, Pham, LeBlanc, Falck, & Inscho, 2000). CYP hydroxylase inhibition or 20-HETE antagonism attenuates endothelin-1 vasoconstriction (Imig et al., 2000). 20-HETE inhibitors also attenuate increases in vascular smooth muscle cell calcium levels in response to endothelin-1 (Imig et al., 2001). Endothelin-1 acts through the endothelin type A (ETA) receptors to increase 20-HETE and vascular smooth muscle cell calcium to cause vasoconstriction (Imig et al., 2001). On the other hand, 20-HETE opposes the vasodilator responses evoked by nitric oxide (Alonso-Galicia et al., 1999). Nitric oxide or superoxide radicals can inhibit vascular 20-HETE generation (Alonso-Galicia et al., 1999). Decreased vascular 20-HETE levels in response to nitric oxide allow for activation of cGMP-independent vasodilation by nitric oxide (Alonso-Galicia et al., 1999). In addition, 20-HETE can act to increase oxidative stress and cause eNOS uncoupling and endothelial dysfunction (Roman, 2002; Williams et al., 2010). Taken as a whole, 20-HETE contributes to the responses to endothelial-derived contracting factor endothelin-1 and opposes dilator responses to endothelial-derived nitric oxide.

As with many biological activities, EETs act as a counterbalance to 20-HETE in regard to vascular responses to the endothelial-derived factors endothelin-1 and nitric oxide. EETs oppose the vasoconstrictor actions of endothelin-1 and have unique interactions with nitric oxide. The epoxygenase inhibitor MS-PPOH enhances endothelin-1 vasoconstriction (Imig et al., 2000). Intriguingly, endothelial expression of human CYP2J2 and CYP2C8 epoxygenases in mice results in attenuated arteriolar constrictor responses to endothelin-1 (Lee et al., 2010). The ability of EETs to oppose endothelin-1 constrictor responses occurs at the endothelial cell because epoxygenase inhibition does not alter endothelin-1-mediated increases in vascular smooth muscle cell calcium (Imig et al., 2000). This finding implies that endothelin-1 increases endothelial generation of EETs to oppose vasoconstriction. In regard to interactions between nitric oxide and EETs, EETs appear to act independent of nitric oxide. On the other hand, endothelial cell EET generation increases in eNOS gene-deficient mice and compensates for the loss of nitric oxide (Huang et al., 2001). This finding demonstrated that EETs minimize the impact of decreased nitric oxide on endothelial cell function and vascular homeostasis. There is also evidence that H₂O₂ can regulate EET bioavailability (Larsen et al., 2008). Evidence has demonstrated that CYP2C9 appears to generate ROS in coronary arteries (Fleming, 2001). CYP2C9 inhibition improves vasodilation and increases nitric oxide levels. Contrary to this finding, EETs can potentiate nitric oxide dilation and EETs increase NOS activity in cultured endothelial cells (Fleming, 2001). Overall, these studies demonstrate endothelial EET generation in response to endothelin-1 opposes vasoconstriction and that interactions between CYP epoxygenases, EETs, nitric oxide, ROS, and eNOS are complex and not fully understood.

These interactions between 20-HETE, EETs, and endocrine, paracrine, and autocrine factors are essential for proper endothelial cell function and vascular homeostasis. This balance is delicate and tightly controlled to maintain overall cardiovascular health. Unfortunately, cardiovascular disease states involve alterations in endocrine, paracrine, and autocrine factors and the ability for CYP eicosanoids to properly maintain endothelial and vascular function. Thus, improper regulation of vascular CYP eicosanoids occurs in cardiovascular disease states and changes in CYP eicosanoids contribute to hypertension, atherosclerosis, stroke, and other cardiovascular diseases.

8. CARDIOVASCULAR DISEASES: HYPERTENSION

Hypertension is a major cardiovascular disease that afflicts one-third of the adult population worldwide. This has led to intensive investigation into the factors contributing to hypertension including CYP eicosanoids. The contribution of CYP eicosanoids to high blood pressure and the associated risk factors has been evaluated in hypertensive animal models as well as in humans (Imig, 2012; Williams et al., 2010). These experimental studies have determined that 20-HETE at the vascular level contributes to hypertension in an androgen-dependent manner (Williams et al., 2010; Wu & Schwartzman, 2011). Decreased endothelial-derived EETs appear to be a contributing factor to increased blood pressure in hypertension (Imig & Hammock, 2009). In many types of hypertension it is an increase in vascular sEH that is responsible for decreasing EET levels (Imig & Hammock, 2009). Intriguingly, EETs and 20-HETE can also influence renal salt transport to influence blood pressure control in hypertension (Imig, 2012; Roman, 2002). In this regard EETs and 20-

HETE increase sodium excretion and are considered to have antihypertensive renal tubular transport actions (Imig, 2012; Roman, 2002). This is in contrast to the EETs and 20-HETE ying/yang actions at the level of the vasculature to influence total peripheral resistance and blood pressure (Imig, 2013). Next, the contribution of CYP eicosanoids to vascular function and blood pressure control in hypertension will be discussed.

20-HETE vascular actions to constrict, increase sensitivity to other vasoconstrictors, enhance angiotensin generation, and interfere with nitric oxide generation are consistent with 20-HETE being prohypertensive (Roman, 2002; Williams et al., 2010). Single nucleotide polymorphisms (SNPs) in CYP4A11 and CYP4F2 enzymes have been linked to increased risk for developing hypertension (Gainer et al., 2005; Williams et al., 2010). There is significant evidence that 20-HETE contributes to angiotensin-dependent hypertension (Wu & Schwartzman, 2011). Human hypertensive patients demonstrate increased 20-HETE levels that correlate with plasma renin activity (Laffer, Laniado-Schwartzman, Wang, Nasjletti, & Eljovich, 2003). 20-HETE inhibition decreases blood pressure when combined with an sEH inhibitor in Ren-2 renin transgenic hypertensive rats (Certikova Chabova et al., 2010). In addition, endothelial expression of CYP4A2 in rats increases ACE activity and AT1 receptor levels to cause angiotensin-dependent hypertension (Cheng et al., 2012). Likewise, vascular dysfunction in the Dahl SS hypertensive rat appears to be due to ROS stimulation of 20-HETE (Lukaszewicz & Lombard, 2013; Williams et al., 2010). Evidence in genetic mice and in rats has demonstrated that increased 20-HETE is a contributor to androgen-dependent hypertension (Holla et al., 2001; Wu et al., 2013). Cyp4a14^{-/-} mice have androgen-mediated hypertension that was a result of increased Cyp4a12 expression and 20-HETE generation (Holla et al., 2001). CYP4A2 transgenic mice demonstrated that human CYP4A2 expression is androgen regulated and that hypertension correlated with 20-HETE levels in these mice (Sodhi et al., 2010; Wu et al., 2013). Even though the contribution of 20-HETE to blood pressure regulation is complex, it is clear that vascular 20-HETE promotes vasoconstriction and vascular dysfunction and contributes to angiotensin II and androgen-dependent hypertension.

Endothelial EET generation has been clearly demonstrated to contribute to many types of hypertension (Imig & Hammock, 2009). Angiotensin-dependent hypertension and SS hypertension have decreased epoxygenase activity or increased sEH activity (Zhao, Pollock, Inscho, Zeldin, & Imig, 2003; Zhao, Yamamoto, et al., 2004). Vascular and kidney CYP2C epoxygenase protein levels are decreased in many types of hypertension (Imig & Hammock, 2009). Likewise, Cyp2c44 genetic-deficient mice develop SS hypertension (Capdevila et al., 2014). Cyp4a10^{-/-} mice demonstrated decreased Cyp2c44 protein expression, decreased EET generation, and hypertension on a normal salt diet (Nakagawa et al., 2006). There is also evidence for decreased EET levels in human hypertension (Bellien & Joannides, 2013; Tacconelli & Patrignani, 2014). Genetic studies have demonstrated associations between CYP epoxygenase enzyme genetic variants and increased risk for hypertension (Bellien & Joannides, 2013; King et al., 2005). Human studies have also demonstrated decreased EET levels contribute to vascular dysfunction (Lee et al., 2011). Genetic variants in EPHX2 that decreases EET levels result in reduced forearm blood flow responses to bradykinin (Lee et al., 2011). Likewise, decreased renal microvascular CYP2C11 and CYP2C23 expression in obese Zucker rats contributes to increased blood pressure (Zhao et al., 2005). Interestingly,

vascular EET levels in obese Zucker rats were further reduced by increased sEH protein expression that contributed endothelial dysfunction (Zhao et al., 2005). Angiotensin-dependent hypertension in rats and mice has also been demonstrated to involve increased vascular sEH protein expression (Imig & Hammock, 2009). Thus, increased sEH and decreased EETs in endothelial cells contribute to the progression of hypertension.

9. CARDIOVASCULAR DISEASES: ATHEROSCLEROSIS AND VASCULAR INFLAMMATION

Atherosclerosis and vascular inflammation are cardiovascular diseases that alterations in CYP eicosanoids contribute importantly to the disease progression. 20-HETE has endothelial cell proinflammatory actions that promote atherosclerosis and vascular remodeling (Hoopes et al., 2015). Proinflammatory endothelial cell changes that occur in response to 20-HETE include increased adhesion molecule expression and cytokine release (Ishizuka et al., 2008). ROS contribute importantly to the vascular inflammatory response to 20-HETE (Ishizuka et al., 2008). 20-HETE increases vascular ROS levels and NF- κ B activity to increase endothelial cell adhesion molecules and IL-8 levels (Ishizuka et al., 2008). These findings clearly demonstrate the important contribution of 20-HETE to endothelial and vascular inflammation.

CYP epoxygenases and sEH have been found to influence vascular inflammation and atherosclerosis (Bellien & Joannides, 2013). EETs have been demonstrated to oppose vascular inflammation and decrease endothelial cell adhesion molecule expression (Imig, 2012). Interestingly, EPHX2 polymorphisms have been linked to risk for coronary artery calcification and disease in the Atherosclerosis Risk in Communities and Coronary Artery Risk Development in young adults studies (Bellien & Joannides, 2013). Experimental studies in EPHX2 gene-deficient mice and using sEH inhibitors have demonstrated a contribution for EETs to oppose vascular inflammation, atherosclerosis, and vascular remodeling (Imig, 2012; Imig & Hammock, 2009). Mice with endothelial expression of CYP2C8 or CYP2J2 or EPHX2 $^{-/-}$ mice have decreased vascular inflammation and NF- κ B activity when exposed to endotoxin (Imig, 2012). EET-positive actions to attenuate atherosclerosis have been associated with decreased adhesion molecules and inflammatory cytokines (Imig, 2012; Node et al., 1999). Thus, EETs and sEH inhibition decrease inflammation and have vascular protective actions that can combat atherosclerosis.

10. CARDIOVASCULAR DISEASES: ISCHEMIC INJURY

The contribution of EETs and 20-HETE to cerebral and cardiac ischemic injury has been extensively investigated. 20-HETE injections into the intracarotid artery can produce cerebral ischemic injury (Roman, 2002; Williams et al., 2010). Likewise, exogenous administration of 20-HETE to coronary arteries increases infarct size in the heart (Roman, 2002; Williams et al., 2010). In agreement with a contribution of 20-HETE to ischemic injury, 20-HETE inhibitors have been demonstrated to reduce cerebral and cardiac ischemia and reperfusion injury (Roman, 2002; Williams et al., 2010). EETs or sEH inhibition protects the brain and heart from damage that occurs following an ischemic event (Alkayed et al., 2002; Imig, 2012; Simpkins et al., 2009). Vasodilator, profibrinolytic, and

antiinflammatory actions potentially contribute to the ischemic protective actions for EETs (Imig, 2012). Epidemiological data demonstrating a genetic polymorphism in the EPHX2 gene are associated with increased risk for ischemic stroke (Bellien & Joannides, 2013). EET, EET analogs, and sEH inhibitors have attenuated cerebral and cardiac ischemic injury (Batchu, Lee, Qadhi, et al., 2011; Imig, 2012). This protective action for EETs appears to be multifactorial and includes actions independent of those on the endothelial or vascular smooth muscle cells (Imig, 2012). EETs likely inhibit apoptosis in the cardiac or brain tissue. Brain and cardiac tissue EET cell signaling antiapoptotic mechanisms involve increased Bcl2, ceramide inhibition, and decreased ROS (Alkayed et al., 2002; Batchu et al., 2011; Simpkins et al., 2009). EETs also sequester MAPKs and activate PI3K pathways in brain and cardiac tissue (Alkayed et al., 2002; Batchu et al., 2011; Simpkins et al., 2009). Other studies have demonstrated cardiac tissue protective actions by delaying or inhibiting opening of the mitochondrial transition pore (Katragadda et al., 2009). As a whole, these findings support the idea that decreasing 20-HETE actions or increasing EET actions would be beneficial in cardiac and cerebral ischemia.

Over the past two decades it has become apparent that CYP eicosanoids contribute importantly to endothelial and vascular alterations that occur during cardiovascular disease states. Experimental studies in cardiovascular disease animal models as well as human cardiovascular disease provide convincing evidence that CYP eicosanoids represent a therapeutic target (Imig & Hammock, 2009; Williams et al., 2010). There has been tremendous progress in the development of CYP eicosanoid therapeutics and novel small-molecule eicosanoid-based drugs are currently in clinical trials. Eicosanoid-based therapeutics will be discussed in the next section.

11. EICOSANOID-BASED CARDIOVASCULAR THERAPEUTICS

There has been excellent progress made in the last 15 years to develop CYP eicosanoid-based therapeutics for cardiovascular diseases. Three approaches have been utilized to advance toward human clinical trials: enzymatic inhibitors, agonists, and antagonists. CYP eicosanoid enzymatic inhibitors have progressed the swiftest because detailed information on protein structure for sEH, hydroxylase, and epoxigenase enzymes is available. Development of 20-HETE and EET agonists and antagonists has required structure-activity relationship studies. 20-HETE and EET agonists and antagonists advancement would be greatly accelerated by binding site/receptor protein identification. Another major hurdle was advancing and modifying promising small molecules for chronic administration in animals and humans. There are now novel small molecules that can be administered chronically that target every CYP-eicosanoid metabolite and pathway.

Novel small molecules that target the CYP hydroxylase and 20-HETE that can be chronically administered are emerging (Williams et al., 2010). A number of selective CYP hydroxylase enzymatic inhibitors have been developed (Williams et al., 2010). Experimental studies have extensively evaluated 20-HETE synthase inhibitors such as DDMS, DDBB, and HET0016 in cardiovascular disease models (Williams et al., 2010). 20-HETE agonists and antagonists have been evaluated in ischemia-reperfusion injury models. Agonists of 20-HETE include 5,14,20-HEDE (WIT003) and 5,14,20-HEDGE. Identifying 20-HETE

antagonists from agonists in the literature is made difficult because 20-HETE antagonists have very similar names like 6,15,20-HEDE (WIT002) and 6,15,20-HEDGE. A 20-HETE antagonist, SOLA, has been chronically administered in the drinking water and demonstrated antihypertensive actions in diabetic and hypertensive rodents (Cheng et al., 2014; Gangadhariah et al., 2015). More importantly, a CYP hydroxylase inhibitor, TS011, has advanced to human clinical trials for neuroprotection in stroke (Williams et al., 2010). Therefore, novel therapeutics that target CYP hydroxylase and 20-HETE could be used to treat human cardiovascular disease in the near future.

Even though progress of epoxygenase inhibitors and EET antagonists is ongoing, a major focus has been on the developing EET agonists for the treatment of cardiovascular diseases. Selective epoxygenase inhibitors like MS-PPOH are readily available and have been utilized in numerous animal studies (Imig, 2012). Regrettably, these selective epoxygenase inhibitors are not ideal for chronic administration and are currently best delivered by an intraperitoneal pump. A major reason for this slow progress is the absence of a clear clinical indication to use these inhibitors. EET antagonists have been developed based on structure-activity relationship studies (Gauthier, Jagadeesh, Falck, & Campbell, 2003). This has resulted in the development of EET antagonists that are selective for 11,12-EET and 14,15-EET (Bukhari et al., 2012). A nonselective EET antagonist, 14,15-EEZE, has been used in animal studies and could be a precursor to an EET antagonist that could be administered orally and chronically (Gauthier et al., 2003). It is likely that such an EET antagonist could be on the horizon given the advanced development of EET agonists. EET agonists have been rapidly developing over the past 5 years because of their potential to treat cardiovascular diseases (Imig, 2012; Khan et al., 2013). NUDSA was one of the first EET analogs to be administered chronically to rodents and demonstrated antihypertensive actions (Imig et al., 2010; Sodhi et al., 2009). Further development of EET analogs led to two orally active novel small molecules, EET-A and EET-B (Hye Khan et al., 2014; Khan et al., 2013). EET-A and EET-B have been utilized in hypertensive and kidney disease animal models (Hye Khan et al., 2014; Khan et al., 2013). These EET analogs have been demonstrated to decrease blood pressure, reduce inflammation, and decrease end-organ damage in cardiovascular disease animal models (Hye Khan et al., 2014; Khan et al., 2013). The therapeutic potential for EET analogs to treat cardiovascular disease will likely be determined in the next decade.

Development of sEH inhibitors as a means to prevent EET conversion to DHETEs has been tested in human clinical trials for cardiovascular diseases (Imig & Hammock, 2009). Utilization was enhanced by the early development of an orally active sEH inhibitor, AUDA (Imig & Hammock, 2009). AUDA and other sEH inhibitors have been tested in cardiovascular disease animal models such as hypertension, metabolic syndrome, and atherosclerosis (Imig & Hammock, 2009). There was a very rapid pace for developing sEH inhibitors for use in humans because these experimental studies demonstrated great potential to treat cardiovascular diseases. This rapid development resulted in testing sEH inhibitors for hypertension and diabetes in initial human clinical trials (Imig & Hammock, 2009). Currently, Phase I clinical trials evaluating sEH inhibitors for chronic obstructive pulmonary disease have been completed ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01762774) Identifier: NCT01762774, 2012). An aspect of these clinical trials is to evaluate sEH inhibitors on forearm blood flow responses

([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01762774) Identifier: NCT01762774, 2012). Thus, the promise for sEH inhibitors to treat cardiovascular disease is progressing and remains an area of active investigation.

12. CONCLUSION

CYP eicosanoid endothelial cell and vascular actions have been extensively studied in physiological and pathological states. These experimental studies have clearly demonstrated opposing endothelial cell actions under the majority of experimental settings for the CYP hydroxylase metabolite 20-HETE with those of CYP epoxygenase EETs. Genetic and pharmacological tools have allowed for extensive evaluation of EET and 20-HETE endothelial cell signaling mechanisms. There have also been numerous studies into CYP eicosanoid interactions with hormones, paracrine factors, and autocrine factors in regard to endothelial cell function and vascular homeostasis. Animal and human experimental studies clearly demonstrate that alteration in CYP eicosanoids at the endothelial and vascular level contributes to cardiovascular diseases. These findings have led to the quest for novel small molecules that act on CYP eicosanoid pathways as therapies for cardiovascular diseases. Accordingly, this is an exciting period for CYP eicosanoids that requires further investigations to reveal new and exciting endothelial cell and vascular actions for the CYP hydroxylase metabolite 20-HETE and CYP epoxygenase EETs.

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ABBREVIATIONS

DHETs	dihydroxyeicosatrienoic acids
EETs	epoxyeicosatrienoic acids
EPC	endothelial progenitor cell
HETEs	hydroxyeicosatetraenoic acids
sEH	soluble epoxide hydrolase

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Fig. 1. Diagram depicting pathways of cytochrome P450 (CYP) arachidonic metabolism. CYP 4A and CYP4F enzymes can generate 20-hydroxyeicosatetraenoic acid (20-HETE). CYP2C and CYP2C enzymes generate epoxyeicosatrienoic acids (EETs). Soluble epoxide hydrolase (sEH) can hydrate EETs to form dihydroxyeicosatrienoic acids (DHETEs).

Fig. 2. Diagram depicting 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) cell signaling mechanisms on vascular tone. 20-HETE inhibits large-conductance K^+ channels and activates L-type Ca^{2+} channels leading to an increase in vascular smooth muscle intracellular Ca^{2+} and subsequent vasoconstriction. Endothelial-derived EETs activate vascular smooth muscle cell large-conductance K^+ channels resulting in hyperpolarization and vasorelaxation.

Fig. 3. Diagram depicting 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) on angiogenesis. Angiogenesis is a biological activity where 20-HETE and EETs act in the same manner. EETs promote angiogenesis through mitogen-activated protein kinase (MAPK) activation and nuclear cyclin D1 generation, sphingosine kinase-1 (SK-1), and phosphatidylinositol 3-kinase (PI3K)/Akt pathways to activate transcription factors and the generation of cell cycle modulators, and protein kinase A (PKA) acting via the cAMP/PKA response element-binding protein (CREBP) resulting in COX-2 production that influences angiogenesis. 20-HETE promotes angiogenesis through vascular endothelial growth factor (VEGF) activation of MAPK/extracellular signaling-regulated (ERK) kinase (MEK) to increase the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and through NADPH oxidase (NOX) and PI3K/Akt pathways.

Fig. 4.

Diagram depicting 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) interactions on endothelial dysfunction. Vascular smooth muscle cell-derived 20-HETE acts on the endothelial cell to decrease nitric oxide (NO), increase angiotensin type 1 receptor (AT1R), and increase angiotensin-converting enzyme (ACE) to generate angiotensin II (ANG II). Increased ANG II and decreased NO will act to promote endothelial dysfunction. On the other hand, bradykinin stimulates endothelial cell EET generation that acts to interfere with ANG II actions on the AT1R and will oppose decreased NO levels resulting in improved endothelial function.