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20-HETE in the regulation of vascular and cardiac function

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Abstract

20-HETE, the ω -hydroxylation product of arachidonic acid catalyzed by enzymes of the cytochrome P450 (CYP) 4A and 4F gene families, is a bioactive lipid mediator with potent effects on the vasculature including stimulation of smooth muscle cell contractility, migration and proliferation as well as activation of endothelial cell dysfunction and inflammation. Clinical studies have shown elevated levels of plasma and urinary 20-HETE in human diseases and conditions such as hypertension, obesity and metabolic syndrome, myocardial infarction, stroke, and chronic kidney diseases. Studies of polymorphic associations also suggest an important role for 20-HETE in hypertension, stroke and myocardial infarction. Animal models of increased 20-HETE production are hypertensive and are more susceptible to cardiovascular injury. The current review summarizes recent findings that focus on the role of 20-HETE in the regulation of vascular and cardiac function and its contribution to the pathology of vascular and cardiac diseases.

Keywords

Cytochrome P450; 20-HETE; Hypertension; Myocardial infarction; Angiogenesis; Vascular remodeling; Heart failure

1. Introduction

Cardiovascular diseases (CVDs) are a group of disorders of the heart and the blood vessels including diseases of the coronary, cerebral and peripheral arteries and diseases of the heart and kidney. CVDs are the number one cause of death globally; more people die annually from CVDs than from any other cause. According to the World Health Organization, an estimated 17.7 million people died from CVDs in 2015 and this number is expected to increase (<http://www.who.int/mediacentre/factsheets/fs317/en/>). 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) has been recognized as an important player in the pathogenesis of CVDs. It is a vasoactive eicosanoid and a key constituent of the cardiovascular system. Its effects in the vasculature are multifaceted and include sensitization of smooth muscle responsiveness to constrictor stimuli, stimulation of cell migration and proliferation, as well as activation of endothelial cell dysfunction and

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Conflict of interest

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inflammation. Such effects have significant implications with regard to the pathophysiology of the cardiovascular system. The clinical relevance of 20-HETE is highlighted by recent studies relating 20-HETE and its biosynthetic enzymes to the development of hypertension, stroke and myocardial infarction, and more recently to obesity and metabolic diseases. This review summarizes findings that focus on the role of 20-HETE in the regulation of vascular and cardiac function and its contribution to the pathology of vascular and cardiac diseases.

2. 20-HETE biosynthesis and action

2.1. Biosynthesis

20-HETE is the ω -hydroxylation metabolite of arachidonic acid and its biosynthesis is catalyzed by cytochrome P450 (CYP) monooxygenases of the 4A and 4F gene subfamily (Hardwick, 2008). In humans, there are two major 20-HETE synthases, CYP4A11 and CYP4F2 (Capdevila, Falck, & Harris, 2000; Lasker et al., 2000; Powell, Wolf, Jin, & Lasker, 1998; Roman, 2002), but others, including CYP4F11 and CYP4F3 have some capacity to produce 20-HETE (Edson & Rettie, 2013). In mice, the primary 20-HETE synthase is Cyp4a12a (Muller et al., 2007) and in the rat the major 20-HETE synthases are CYP4A1, 2 and 3 (Hardwick, 2008; Nguyen, Wang, Reddy, Falck, & Schwartzman, 1999) as well as CYP4F1 and 4 (Imaoka, Hashizume, & Funae, 2005; Joseph et al., 2017; Soler et al., 2018; Xu, Falck, Ortiz de Montellano, & Kroetz, 2004). These enzymes are expressed in different tissues and cell types and are subjected to transcriptional regulation by nuclear receptor and transcriptional factors, which are activated by hormones such as androgens and by xenobiotics. CYP enzymes are also subjected to epigenetic regulation (e.g., DNA methylation and histone acetylation) and post translational modification (Tang & Chen, 2015). However, no information is available regarding epigenetic or posttranslational regulation of members of the CYP4A/F gene families which are the 20-HETE producing enzymes. MicroRNAs (miR), endogenous 22-nucleotide non-coding RNAs that regulate gene expression via translational repression and degradation of RNAs, have also been shown to regulate CYP enzymes (Nakano & Nakajima, 2018; Shahabi, Siest, Meyer, & Visvikis-Siest, 2014). As for 20-HETE-producing CYPs, a recent study implicated miR-145 as an upstream regulator of cardiac 20-HETE production in metabolic syndrome rats; administration of adenoviral vector carrying miR-145 effectively lowered levels of CYP4F protein and 20-HETE in the ischemic zone of metabolic syndrome rats undergoing transient, repetitive ischemia (Joseph et al., 2017). The existence of several pathways by which the expression and the activity of 20-HETE-producing enzymes can be regulated implies that individual variations in CYP4A/F may not only be due to SNPs but also may arise from post transcriptional modifications. While much has been done with respect to identification of genetic variants in CYP4A11 and CYP4F2, the major human 20-HETE synthases (Fava, Ricci, Melander, & Minuz, 2012; Wu, Gupta, Garcia, Ding, & Schwartzman, 2014; Zordoky & El-Kadi, 2010), not much is known regarding their regulation at the post transcription levels; an area that is yet to be explored.

20-HETE formation has been identified in the liver, kidney, heart, lung, conductive and resistant arterial vessels, brain, small intestine and pancreas (Capdevila, Falck, & Estabrook, 1992; Joseph et al., 2017; Macica, Balazy, Falck, Mioskowski, & Carroll, 1993;

Schwartzman et al., 1990; Tunaru et al., 2018; Yousif, Benter, & Roman, 2009; Zhu, Zhang, Medhora, & Jacobs, 1985). One of the major sites of 20-HETE biosynthesis within many of these tissues is the blood vessel wall. The smooth muscle layer constitutes the primary site of 20-HETE synthesis. (Gebremedhin et al., 1998; Harder et al., 1994; Miyata & Roman, 2005). However, there are reports that demonstrate significant CYP4A/F expression in the vascular endothelium, especially under ischemic or hypoxic conditions, suggesting that the endothelium has the capacity to produce 20-HETE (L. Chen et al., 2016; Joseph et al., 2017; Zhu et al., 1985). In addition, peripheral blood myeloid and bone marrow-derived cells such as neutrophils, platelets and endothelial progenitor cells (EPC) are also sources of 20-HETE that may contribute to circulatory levels of 20-HETE and to its levels in inflamed and injured tissues (L. Chen et al., 2014; Christmas et al., 2001; Hill & Murphy, 1992; Rosolowsky, Falck, & Campbell, 1996).

20-HETE biosynthesis and its levels are controlled by enzyme expression and activity as well as by factors such as substrate and cofactor availability. Vasoactive autacoids including angiotensin II (Ang II), endothelin-1 and norepinephrine increase 20-HETE production by stimulating the release of arachidonic acid from phospholipids (Carroll et al., 1997; Croft, McGiff, Sanchez-Mendoza, & Carroll, 2000; Hercule & Oyekan, 2000; Lima, Yanes, Davis, & Reckelhoff, 2013; Ljuca & Drevensek, 2010; Speed, George, Arany, Cockrell, & Granger, 2011; Tsai, Croft, Puddey, Beilin, & Barden, 2011). In the kidney, parathyroid hormone (PTH) and epidermal growth factor (EGF) have been shown to stimulate 20-HETE release presumably via increasing substrate availability (Lin, Rios, Falck, Belosludtsev, & Schwartzman, 1995; Omata, Abraham, & Schwartzman, 1992; Ribeiro, Dubay, Falck, & Mandel, 1994). Androgen is a transcriptional activator of CYP4 enzymes. In rats and mice, increased androgen levels or administration of 5 α -dihydrotestosterone (DHT) induce CYP4A enzymes and 20-HETE biosynthesis, creating experimental models of 20-HETE-dependent hypertension (Ding et al., 2013; Holla et al., 2001; Liu, Wu, Liu, Lai, & Zhao, 2012; Nakagawa, Marji, Schwartzman, Waterman, & Capdevila, 2003; Singh et al., 2007; Wu et al., 2013; Wu & Schwartzman, 2011). Renal production of 20-HETE is higher in male spontaneously hypertensive rats (SHR) than female SHR or male WKY rats; castration or administration of androgen receptor antagonists significantly lowers renal CYP4A proteins and 20-HETE levels in male SHR (Ishizuka, Ito, Omata, & Ito, 2004). A study by Dalmaso et al (Dalmaso et al., 2016) showed that DHT administered to female SD rats increased blood pressure and was associated with increased renal microvascular CYP4A2 expression and renal production of 20-HETE. Estrogen and growth hormone have also been shown to increase CYP4A/F expression primarily in non vascular tissues (Kalsotra, Anakk, Boehme, & Strobel, 2002; Y. Zhang & Klaassen, 2013). In ovariectomized SHR, a model of postmenopausal hypertension, treatment with estrogen alone increased aortic CYP4F3 expression but not 20-HETE production. Addition of testosterone to the estrogen treatment potentiated CYP4F3 expression and significantly increased 20-HETE production in the aorta (Costa et al., 2018).

Other factors that influence 20-HETE production include carbon monoxide (CO), nitric oxide (NO) and superoxide (<mi>). CO and NO bind to the heme moiety of the CYP enzymes and inactivate them and both have been shown to inhibit 20-HETE production (Alonso-Galicia, Hudetz, Shen, Harder, & Roman, 1999; Minamiyama et al., 1997; Oyekan,

Youseff, Fulton, Quilley, & McGiff, 1999; Sun et al., 1998; M.H. Wang et al., 2003). The inhibitory actions of NO and CO on CYP enzymes have been shown to influence 20-HETE bioactions. For example, induction of heme oxygenase results in decreased 20-HETE production (Botros, Laniado-Schwartzman, & Abraham, 2002; DaSilva et al., 1994; Levere, Martesak, Escalante, Schwartzman, & Abraham, 1990; Sabaawy et al., 2001; Sacerdoti et al., 1989). The CO vasodilatory action has been attributed, in part, to its ability to inhibit production and counteract constrictor activity of 20-HETE (Johnson, Lavesa, Askari, Abraham, & Nasjletti, 1995; J. Kaide et al., 2004; J. I. Kaide et al., 2001; Kozma et al., 1999). The cGMP-independent component of NO vasodilatory effect in renal and cerebral microcirculation has been attributed to inhibition by NO of 20-HETE production (Sun, Falck, Okamoto, Harder, & Roman, 2000; Yu, Sun, Maier, Harder, & Roman, 2002). In animals treated with NO inhibitor, the resulted hypertension and end organ damage in the kidney is associated with increased CYP4A expression and 20-HETE bio-synthesis (Benter et al., 2005; Hercule, Wang, & Oyekan, 2003; A. O. Oyekan et al., 1999; Yousif & Benter, 2010). Superoxide has also been shown to affect 20-HETE levels. Gebremedhin et al (Gebremedhin, Yamaura, & Harder, 2008) showed that in cerebral arterial smooth muscle cells, exposure to hypoxia results in generation of superoxide which was associated with reduction in 20-HETE levels and biosynthesis. Others showed that chronic, but not acute (Hoagland, Maier, & Roman, 2003), administration of Tempol suppressed CYP4A protein expression in cerebral arteries from high salt fed Dahl SS rats and suggested that this suppression is mediated via restoration of NO production (Lukaszewicz, Paudyal, Falck, & Lombard, 2016). As NADPH is required for 20-HETE's biosynthesis, any manipulations in its cellular levels could results in alteration in 20-HETE levels. A recent study showed that pharmacological inhibition or knockdown of glucose 6-phosphate dehydrogenase (G6PD), a major NADPH producing enzyme, abrogates 20-HETE production in pulmonary arteries (Lakhkar et al., 2016). Interestingly, proteomic analysis by LC-MS/MS in bovine pulmonary arteries detected complexes of G6PD with CYPs suggesting that these two enzymatic systems are functionally coupled (Lakhkar et al., 2016).

2.2. Bioactions in the vasculature

20-HETE has a wide spectrum of biological activities; most applicable to this review are its actions in the vasculature. 20-HETE is a vasoactive lipid molecule and a prominent constrictor of microcirculatory districts where its production is prominent. Its effects on vascular function are multifaceted and include stimulation of smooth muscle contractility, migration and proliferation, as well as modulation of endothelial cell dysfunction and inflammation (Fan et al., 2016; Hoopes, Garcia, Edin, Schwartzman, & Zeldin, 2015; Miyata & Roman, 2005). Such effects may have significant implications with regard to the regulation of the cardiovascular system and the development of CVDs.

2.2.1. 20-HETE effects on vascular smooth muscle cells—In vascular smooth muscle cells (VSMC), 20-HETE stimulates contractions via mechanisms that include activation of protein kinase C (PKC), mitogen-activated protein kinase (MAPK) and Src-type tyrosine kinases (c-Src), all of which can phosphorylate and inhibit the Ca^{2+} -activated K^+ channels (BK_{ca}), leading to depolarization, elevation in cytosolic $[\text{Ca}^{2+}]$, and increased Ca^{2+} entry through the L-type Ca^{2+} channels (Fan et al., 2016; Williams, Murphy, Burke, &

Roman, 2010). This inhibitory action is believed to underlie the effect of 20-HETE in enhancing responsiveness of arterial vessels to constrictor stimuli including pressure (Kausser et al., 1991), oxygen (Harder et al., 1996; Kerkhof, Bakker, & Sipkema, 1999), phenylephrine (J. Kaide et al., 2003; F. Zhang et al., 2001), and endothelin (Oyekan, Balazy, & McGiff, 1997), as well as inhibition of NO synthesis (Sun et al., 1998). 20-HETE also acts through Rho kinase to maintain the phosphorylated state of myosin light chain (MLC20) and increase the sensitivity of the contractile apparatus to Ca^{2+} (Randriamboavonjy, Busse, & Fleming, 2003). Overexpression of CYP4A-20-HETE in vitro by plasmid CYP4A2 cDNA transfection increases while downregulation in vivo by antisense oligonucleotides attenuates vascular reactivity to constrictor stimuli (J. Kaide et al., 2003; M. H. Wang et al., 2001; F. Zhang et al., 2004). 20-HETE's ability to promote VSMC contraction may contribute to the hypertension seen in experimental models with elevated levels of 20-HETE (Imig, Falck, Gebremedhin, Harder, & Roman, 1993; Inoue et al., 2009; Nakagawa et al., 2003; Singh et al., 2007; Wu et al., 2013; F. Zhang et al., 2001).

The transient receptor potential (TRP) (TRPC) and vanilloid (TRPV) receptor family of Ca^{2+} -permeable channels are ion channels, which have a central role in Ca^{2+} homeostasis in virtually all cells, are associated with vascular tone regulation and remodeling and are activated primarily by intracellular ligands (Benham, Davis, & Randall, 2002). 20-HETE has been shown to activate the TRPC6, a non-voltage-gated Ca^{2+} entry/depolarization channel (Basora, Boulay, Bilodeau, Rousseau, & Payet, 2003). A study by Inoue et al (Inoue et al., 2009) showed that 20-HETE production is essential for mechanical potentiation of TRPC6 transmembrane Ca^{2+} mobilization in vascular smooth muscle. Dysregulation of the 20-HETE/TRPC6 pathway contributes to maladaptation of aged cerebral arteries to hypertension (Toth et al., 2013; Toth et al., 2013). Interestingly, TRPC6 can be activated via $\text{G}_{q/11}$ receptors by mechanisms downstream phospholipase C (Trebak, Lemonnier, Smyth, Vazquez, & Putney Jr., 2007). In view of the recent identification of GPR75, a $\text{G}_{q/11}$ GPCR, it is possible that 20-HETE activates TRPC6 following its binding to its GPCR receptor. 20-HETE has also been shown to activate TRPV1 in neurons innervating the vasculature in a kinase-dependent manner (Wen et al., 2012). Scotland et al (Scotland et al., 2004) showed that myogenic response to intraluminal transmural pressure or constriction to 20-HETE in mesenteric small arteries is markedly suppressed following ablation of sensory C-fiber activity or capsazepine (TRPV1 blocker); they further suggested that elevation of intraluminal pressure stimulates generation of 20-HETE which, in turn, activates TRPV1 on C-fiber nerve endings resulting in depolarization of nerves and consequent vasoactive neuropeptide release. Bubb et al (2013) demonstrated that 20-HETE causes dose-dependent increases in blood pressure, coronary perfusion pressure (isolated Langendorff), and pressure-induced constriction of resistance arteries (perfusion myography) that is substantially attenuated in TRPV1 knockout mice. These authors suggested the presence of a vasoconstrictor 20-HETE/TRPV1 pathway that may offer potential for therapeutic targeting in conditions such as hypertension (Bubb et al., 2013).

In addition to its effect on VSMC contractility, 20-HETE has been shown to stimulate VSMC migration via the MAPK-PI3K (D. Stec, Gannon, Beaird, & Drummond, 2007) and proliferation via the RAS/MAPK (Muthalif et al., 2001) pathway, which may contribute to remodeling in hypertension (D. Stec, Gannon, Beaird, & Drummond, 2007; Yaghini et al.,

2005) and to intimal hyperplasia and vascular remodeling after endothelial injury (Orozco et al., 2013; Yaghini et al., 2005). 20-HETE has also been shown to stimulate superoxide production and increase levels of inflammatory cytokines in smooth muscle cells. A recent study by Lakhkar et al. (Lakhkar et al., 2016) demonstrated that 20-HETE stimulates mitochondrial superoxide production and promotes the inflammatory and synthetic phenotype of pulmonary artery vascular smooth muscle cells, potentially contributing to vascular remodeling in pulmonary hypertension. Interestingly, the same group reported that 20-HETE repressed miR-143, which is known to suppress the synthetic phenotype of smooth muscle cells.

2.2.2. 20-HETE effects on the vascular endothelium—The vascular endothelium, which in most circulatory districts lack significant capacity to produce 20-HETE, is a major cellular target for 20-HETE bioactions. However, the assertion that 20-HETE is only produced by the smooth muscle has been recently questioned as reports suggest that endothelial CYP4A-20-HETE synthase is induced under conditions of hypoxia/ischemia (L. Chen et al., 2014; L. Chen et al., 2016; Joseph et al., 2017). In cultured human endothelial cells, hypoxia (3% O₂) for 16 h increases 20-HETE levels by 10-fold (L. Chen et al., 2016). These findings raise the possibility of multiple cellular sources of 20-HETE within the vasculature from which 20-HETE may act on the vascular endothelium including, VSMC, neutrophils and potentially injured endothelial cells. This possibility needs to be further examined.

Studies from our laboratory (Cheng et al., 2008; Singh et al., 2007; J. S. Wang et al., 2006) and others (Dunn et al., 2008; Frisbee, Falck, & Lombard, 2000) using cultured endothelial cells and isolated blood vessels demonstrated that in the systemic circulation (excluding the pulmonary (Yu et al., 2002)), 20-HETE impairs endothelial function through uncoupling of endothelial nitric oxide synthase (eNOS) (Cheng et al., 2008; Ward, Chen, Li, Croft, & Keaney Jr., 2011), stimulation of superoxide production (Cheng et al., 2008; Guo et al., 2007; Medhora et al., 2008), and activation of the NF- κ B-mediated pro-in-inflammatory program (Cheng et al., 2010; Ishizuka et al., 2008). In vivo, elevation of 20-HETE levels is associated with endothelial dysfunction (Inoue, Sodhi, et al., 2009; Singh et al., 2007; J. S. Wang et al., 2006). Inhibition of 20-HETE's actions with 20-SOLA, restored endothelial function and survival in metabolic syndrome rats that display endothelial dysfunction in vivo (Joseph et al., 2017). The action of 20-HETE on eNOS may underlie its ability to impair relaxation and contributes to imbalance of vascular hemostasis. Studies in our laboratory demonstrated impaired relaxation to acetylcholine in arteries from DHT-treated mice that was associated with a marked decrease in the levels of phosphorylated-S239 VASP (vasodilator-stimulated phosphoprotein), an indicator of bioactive NO and the activity of the NO/cGMP/PKG signaling pathway (Oelze et al., 2000); the impaired relaxation and the diminished eNOS-NO pathway were prevented when DHT-treated mice were treated with 20-HETE biosynthesis inhibitor, suggesting that endogenous 20-HETE contributes to endothelial dysfunction in vivo (Cheng et al., 2008). The relationship between 20-HETE, endothelial dysfunction and oxidative stress was also suggested in clinical studies. These studies showed positive correlations between urinary or plasma 20-HETE, oxidative stress and endothelial dysfunction in human subjects (Barden et al., 2007; Ward et al., 2004; Ward,

Puddey, Hodgson, Beilin, & Croft, 2005). Schuck et al (Schuck et al., 2013) examined relationship between CYP-derived eicosanoids and vascular function phenotypes in 106 patients with stable, angiographically-confirmed coronary artery disease (CAD). They found a significant inverse relationship between plasma 20-HETE and brachial artery flow-mediated dilation, further suggesting that despite being treated (e.g., ACE inhibitors), CAD patients with highest 20-HETE levels may be susceptible to more advanced endothelial dysfunction and may benefit from therapies that include 20-HETE inhibition (Schuck et al., 2013).

It is interesting to note that unlike other vascular beds, pulmonary arteries dilate in response to 20-HETE via eNOS activation and increased NO production (Chen, Medhora, Falck, Pritchard, & Jacobs, 2006; Jacobs, Zhu, Gruenloh, Lopez, & Medhora, 2006). This group of investigators further showed that in bovine pulmonary artery endothelial cells 20-HETE increases NO production by stimulating NADPH oxidase-dependent formation of superoxide that rapidly dismutated to hydrogen peroxide, which then mediates activation of phosphoinositide 3-kinase (PI3-kinase)/protein kinase B (Akt)-mediated phosphorylation of eNOS and enhanced release of NO from activated eNOS (Bodiga et al., 2010). These apparent contrasting bioactions of 20-HETE may be the consequence of activation of distinct receptors or different signaling cascades.

20-HETE is a potent inducer of endothelial angiotensin converting enzyme (ACE) expression and activity. It activates ACE transcription and increases ACE activity via stimulation of NF- κ B binding to the ACE promoter (Cheng et al., 2012; Garcia, Shkolnik, Milhau, Falck, & Schwartzman, 2016). Transcriptional activation of endothelial ACE by 20-HETE is mediated via a distinct epidermal growth factor receptor (EGFR)/MAPK/inhibitor of kappa B kinase (IKK β) signaling cascade culminating with increased ACE protein expression and activity (Garcia et al., 2016). Moreover, increased synthesis of 20-HETE in vivo is accompanied by increased vascular ACE expression (Garcia et al., 2014; Garcia et al., 2015) and circulating Ang II levels (Sodhi et al., 2010b). In these studies, administration of a 20-HETE biosynthesis inhibitor or 20-HETE antagonist prevented the increased expression of ACE and decreased circulating Ang II levels, suggesting a role for 20-HETE in the regulation of ACE expression and AngII levels in vivo.

20-HETE promotes endothelial activation and inflammation that involves the secretion of inflammatory cytokines (IL-6, IL-8, TNF α) (Cheng et al., 2014; Ishizuka et al., 2008; Lakhkar et al., 2016) and expression of adhesion molecules (ICAM/VCAM) (Cheng et al., 2014; Guo et al., 2007; Guo et al., 2009; Ishizuka et al., 2008). Inhibition of 20-HETE attenuates vascular inflammation shown by inhibition of NF κ B activation and reduced expression of inflammatory cytokines (Toth et al., 2013). Interestingly, Schuck et al (Schuck et al., 2013) demonstrated a positive relationship between plasma 20-HETE levels and circulatory cellular adhesion molecules in patients with stable CAD, suggesting that patients with the highest levels of 20-HETE may be predisposed to more advanced vascular inflammation. 20-HETE also affect endothelial cell proliferation and survival. It protects against apoptosis and increases cell survival in bovine pulmonary artery endothelial cells through activation of PI3-kinase and Akt pathways and generation of NADPH-derived superoxide (Dhanasekaran et al., 2009). On the other hand, elevated 20-HETE levels are

associated with impaired survival of coronary artery endothelium (Joseph et al., 2017). These studies suggest specificity of actions dependent on the vascular bed involved.

The pro-angiogenic property of 20-HETE was firstly suggested by Amaral et al demonstrating that electrical stimulation induced angio-genesis in skeletal muscle and was abrogated by inhibitor of 20-HETE biosynthesis (Amaral, Maier, Schippers, Roman, & Greene, 2003). Later, a study by Jiang et al, documented the ability of 20-HETE to stimulate endothelial cell angiogenesis in vitro; the same study also showed that 20-HETE generated in the vascular smooth muscle stimulated endothelial sprouting and capillary formation in blood vessel explants ex vivo (Jiang et al., 2004). The mechanism by which 20-HETE exerts its angiogenic activity has been studied by Guo and colleagues (Chen, Ackerman, & Guo, 2012; Guo et al., 2007; Guo et al., 2009) primarily with regard to functions of endothelial progenitor cells (EPC) in the process of angiogenesis. They showed that 20-HETE stimulates the proliferation, migration and tube formation of endothelial progenitor cells (EPC) as well as secretion of proangiogenic molecules (such as HIF-1 α , VEGF, SDF-1 α). They further showed that these effects were mediated through c-Src and EGFR and their downstream signaling pathways, including MAPK and PI3K/Akt pathways, eNOS uncoupling, and NADPH oxidases/reactive oxygen species (ROS) system activation. This signaling cascade is thought to be the mechanism underlying 20-HETE contribution to ischemia-induced neovascularization (L. Chen et al., 2016). Similar findings were reported in endothelial cells derived from Tie-CYP4F2 overexpressing transgenic mice showing that CYP4F2-derived 20-HETE mediates EC proliferation and angiogenesis in a VEGF- and NADPH oxidase-dependent manner (Cheng et al., 2014).

2.3. 20-HETE receptor

The aforementioned biological activities of 20-HETE in the vasculature are manifested at concentrations ranging from low picomolar to high nanomolar, are mediated through activation of second messenger systems, and are 20-HETE-specific as structurally related endogenous lipids, e.g., 19-HETE, 12-HETE, do not exert the same effects and chemically-related 20-HETE analogs mimic or block the bioactions of 20-HETE. These characteristics implicate the existence of a binding site or receptor for 20-HETE. The quest for such a receptor began with the first demonstration that it is a bioactive lipid; however, as a fatty acid, 20-HETE readily crosses membranes and esterifies into phospholipids and other complex lipid molecules. These features hindered studies aimed at identifying the 20-HETE's receptor(s). The action of 20-HETE in endothelial cells, which was extensively studied, provided an excellent foundation for receptor exploration. Our laboratory identified the phosphorylation of EGFR as the first step in the MAPK-IKK β -NF- κ B signaling cascade, leading to ACE induction, eNOS uncoupling and inflammatory cytokine production (Cheng et al., 2008; Cheng et al., 2010; Cheng et al., 2012; Ishizuka et al., 2008). The question we posed was how did 20-HETE activate EGFR phosphorylation? We postulated that as a small lipid molecule, 20-HETE could cross the cell membrane and stimulate tyrosine kinases within the intracellular milieu. Alternatively, 20-HETE might interact with a G-protein coupled receptor (GPCR), the activation of which could lead to transactivation of the EGFR, as was documented for many autacoids including Ang II (Mehta & Griendling, 2007; Okada, 2012) and proposed by others (Akbulut et al., 2009).

In a recent publication, we reported the identification of GPR75, an orphan G-protein ($G_{q/11}$) coupled receptor (GPCR), as a specific cellular target to which 20-HETE binds and through which it activates a signaling cascade that culminates in many of the functional outcomes attributed to 20-HETE *in vitro* and *in vivo* (Garcia et al., 2017). GPR75 has been shown to be expressed in a wide variety of tissues including the brain, heart and kidney. All of these tissues have been shown to have the capacity to produce and to respond to 20-HETE. The binding of 20-HETE to GPR75 elicits distinct signaling pathways in vascular endothelial and smooth muscle cells. In endothelial cells, 20-HETE binding to GPR75 stimulated $G_{q/11}$ protein dissociation and increased inositol phosphate (IP-1) accumulation as well as GPCR-kinase interacting protein-1 (GIT1)-GPR75 binding, which further facilitated the c-Src-mediated transactivation of endothelial EGFR. This results in downstream signaling pathways which induce ACE expression and endothelial dysfunction. Knockdown of GPR75 or GIT1 prevented 20-HETE-mediated EGFR phosphorylation and ACE induction. In VSMC, GPR75–20-HETE pairing is associated with $G_{q/11}$ - and GIT1-mediated PKC-stimulated phosphorylation of MaxiK β , linking GPR75 activation to 20-HETE-mediated vasoconstriction. Furthermore, GPR75 knockdown in a mouse model of 20-HETE-dependent hypertension prevented blood pressure elevation and 20-HETE-mediated increases in ACE expression, endothelial dysfunction, smooth muscle contractility and vascular remodeling. The discovery of 20-HETE-GPR75 pairing provides the molecular basis for the signaling and pathophysiological functions mediated by 20-HETE in hypertension and CVDs as postulated in Fig. 1.

A recent study by Tunaru et al., reported that 20-HETE is an agonist of the long-chain fatty acid receptor FFAR1, a $G_{q/11}$ -coupled GPCR which is also known as GPR40 (Tunaru et al., 2018). They showed that 20-HETE was more efficacious than dietary free fatty acids in activating FFAR1 when tested for induction of Ca^{2+} transients and in promoting glucose-stimulated insulin secretion in pancreatic β -cells. However, 20-HETE activated this receptor at micromolar to millimolar concentrations; much higher than the reported concentrations of 20-HETE that activate GPR75 (Garcia et al., 2017) and exert biological activities such as vasoconstriction and endothelial dysfunction (Garcia & Schwartzman, 2017; Hoopes et al., 2015). It is possible that the binding of 20-HETE to FFAR1, the expression of which is limited to the pancreatic α and β cells and to the enteroendocrine cells within the intestine, becomes functionally relevant under conditions of high levels of 20-HETE. Certainly, the possibility of multiple receptors for this lipid mediator should be further investigated especially under pathological conditions and with regard to specific cells and tissues.

3. 20-HETE and vascular function

3.1. Vascular tone and blood pressure

The ability to stimulate smooth muscle contraction and impair endothelial-derived vasodilation suggests that 20-HETE may play a major role in the regulation of vascular tone and in the control of blood pressure. Extensive studies by Roman and Harder groups showed that 20-HETE is a key determinant of myogenic response and autoregulation of cerebral and renal blood flow (Gebremedhin et al., 2000; Harder, Campbell, & Roman, 1995; Harder, Narayanan, & Gebremedhin, 2011; Imig, Zou, Ortiz de Montellano, Sui, & Roman, 1994;

Zou et al., 1994). Elevated production of 20-HETE in SHR contributes to increased renal vascular tone (Dunn et al., 2008; Gebremedhin, Ma, Imig, Harder, & Roman, 1993; Imig et al., 1993) whereas genetic deficiency of 20-HETE biosynthesis in Dahl salt-sensitive (SS) rats contributes to impaired myogenic tone (Fan et al., 2015; Ge et al., 2014; Lukaszewicz & Lombard, 2013; Ren, D'Ambrosio, Garvin, Peterson, & Carretero, 2014). Transfection of CYP4A1, a 20-HETE synthase, increases vascular reactivity of renal interlobar arteries as well as decreases diameter and increases responsiveness of gracilis muscle arteriols to pressure in a 20-HETE-dependent manner (F. Zhang et al., 2004). In contrast, administration of CYP4A1 antisense oligonucleotide reduces vascular reactivity of mesenteric arteries (M. H. Wang et al., 2001). Increased vascular tone and peripheral vascular resistance have been documented in all animal models of 20-HETE overproduction, including the SHR, androgen-treated rats and mice, endothelial-specific CYP4A2-transduced rats, Cyp4a14 null mice and Cyp4a12 transgenic mice; in all, administration of either 20-HETE biosynthetic inhibitors such as HET0016 or 20-HETE antagonists (20-6,15-HEDE, 20-SOLA) prevented the increases in vascular tone. Moreover, models of 20-HETE overproduction display 20-HETE-dependent endothelial dysfunction that may further contribute to the regulation of vascular tone by 20-HETE (Inoue, Sodhi, et al., 2009; Singh et al., 2007; J. S. Wang et al., 2006).

The bioactions of 20-HETE in the vasculature including vasoconstriction and endothelial dysfunction may underlie, in part, its ability to promote the development of hypertension. The link between 20-HETE and hypertension was originally documented in studies indicating that depletion and inhibition of CYP4A reduced blood pressure in the SHR (DaSilva et al., 1994; Escalante, Sacerdoti, Davidian, Schwartzman, & McGiff, 1991; Sacerdoti et al., 1989). These and other studies (Su, Kaushal, & Kroetz, 1998; Xu et al., 2002), together with reports that 20-HETE is a vasoconstrictor (Escalante et al., 1993; Laniado-Schwartzman, Falck, Yadagiri, & Escalante, 1989; Ma et al., 1993), suggested that increased 20-HETE synthesis and/or actions contributed to increased blood pressure in SHR and other experimental models of hypertension (Alonso-Galicia, Maier, Greene, Cowley Jr., & Roman, 2002; Moreno, Maier, Hoagland, Yu, & Roman, 2001; Muthalif et al., 2000; Muthalif et al., 2000)(30–33). Other studies substantiated the notion that 20-HETE contributes to the development of hypertension by demonstrating that: (i) the synthesis of and vascular reactivity to 20-HETE are significantly higher in the SHR than in normotensive rats (Imig et al., 1993; F. Zhang et al., 2001), (ii) inhibition of vascular 20-HETE synthesis by CYP4A2 antisense oligonucleotides decreases blood pressure (M. H. Wang et al., 1998; M. H. Wang et al., 2001), (iii) vascular-selective transduction of CYP4A2 cDNA increases vascular 20-HETE synthesis and produces hypertension in SD rats (Inoue, Sodhi, et al., 2009; J. S. Wang et al., 2006), and (iv) administration of a 20-HETE analog prevents the fall in blood pressure in LPS-induced endotoxic shock in rats (Tunctan et al., 2008). Androgen-induced hypertension in male and female SD rats is 20-HETE-dependent (Nakagawa et al., 2003; Singh et al., 2007; Singh & Schwartzman, 2008). Interestingly, a study by Dalmaso et al (2016) demonstrated that administration of DHT to Dahl salt-resistance female rats increases blood pressure but it did not affect blood pressure when administered to Dahl salt-sensitive female rats, which are known to be 20-HETE-deficient (Ma, Schwartzman, & Roman, 1994), supporting the notion that 20-HETE mediates androgen-dependent

hypertension. The male-specific hypertension displayed by the Cyp4a14 knockout mouse is driven by androgen-dependent induction of Cyp4a12 expression (the mouse homologue of CYP4A8) and increases in 20-HETE synthesis (Holla et al., 2001; Wu et al., 2013). Indeed, the conditional Cyp4a12 overexpressed mice are hypertensive when placed on doxycycline (Wu et al., 2013). Mice overexpressing human CYP4F2, which has been associated with hypertension and CVDs in human (Deng et al., 2010; Fava et al., 2008; Fu et al., 2008; Fu et al., 2009; H. Liu et al., 2008), exhibit enhanced 20-HETE production and elevated blood pressure (X. Liu et al., 2009). Overall, in all models, hypertension was correlated with increases in 20-HETE production, vascular contractility and endothelial dysfunction. More importantly, the increase in blood pressure was readily prevented and reversed by 20-HETE inhibitors or 20-HETE antagonists, strongly implicating 20-HETE as the driving force for the hypertension. (Garcia et al., 2015; Inoue, Sodhi, et al., 2009; Singh et al., 2007; Sodhi et al., 2010a; J. S. Wang et al., 2006; Wu et al., 2011; Wu et al., 2013)

The finding that 20-HETE induces ACE added another mechanism by which 20-HETE promotes hypertension. As indicated above, 20-HETE activates ACE transcription and increases ACE activity via stimulation of NF- κ B binding to the ACE promoter (Cheng et al., 2012; Garcia et al., 2016). Hence, increased vascular synthesis of 20-HETE *in vivo* is associated with increased ACE expression and circulating Ang II levels (Sodhi et al., 2010b), and, in models of 20-HETE-driven hypertension, ACE inhibitors or AT₁R blockers prevented and reversed the blood pressure elevation (Garcia et al., 2014; Garcia et al., 2015) suggesting a close interaction between 20-HETE and the RAS in which ACE induction by 20-HETE brings about an increase in Ang II which, in turn and together with 20-HETE, promotes blood pressure elevation.

The contribution of 20-HETE to hypertension is complex due to its actions in the kidney; 20-HETE inhibits the Na⁺-K⁺-2Cl⁻ co-transporter in the thick ascending loop of Henle's and therefore contributes to Na⁺ excretion (Escalante, Erlj, Falck, & McGiff, 1994; Nowicki et al., 1997). Inhibition of 20-HETE synthesis in the medullary thick ascending limbs and outer medullary regions (Stec, Mattson, & Roman, 1997) has been shown to increase blood pressure and sodium reabsorption in Lewis rats. Stimulation of the renal formation of 20-HETE with clofibrate, a peroxisome proliferator-activated receptor-agonist that upregulates CYP4A, lowers blood pressure in Dahl salt-sensitive (Roman, Ma, Frohlich, & Markham, 1993), deoxycorticosterone acetate (DOCA)-salt and high-fat-induced hypertensive rats (Zhou et al., 2006). The importance of reduction in outer medullary renal 20-HETE and its contribution to salt-sensitive hypertension was further supported by Stec et. al., who crossed the Dahl salt-sensitive (SS/Jr) rats, which exhibit decreased CYP4A2 protein levels in the outer medulla, with the Lewis strain. The resulting F₂ progeny demonstrated that the CYP4A2 allele co-segregates with the development of salt-induced hypertension (Hoagland et al., 2004; D. E. Stec, Mattson, & Roman, 1996; Stec, Trolle, Krieger, Jacob, & Roman, 1996). Williams et al. also showed that the transfer of chromosome 5 containing the CYP4A genes from Brown Norway (BN) rats on the Dahl S salt-sensitive (SS) genetic background increases the renal expression of CYP4A protein and the production of 20-HETE and that 20-HETE contributes to the antihypertensive and renoprotective effects seen in the SS.5 (BN) consomic strain (Williams et al., 2008; Williams et al., 2012). Also, in humans a deficiency in 20-HETE synthesis and/or action, measured as decreased urinary excretion in

response to salt loading, has been linked to salt-sensitive hypertension (Laffer et al., 2008; Laffer, Laniado-Schwartzman, Wang, Nasjletti, & Eljovich, 2003a; Laffer, Laniado-Schwartzman, Wang, Nasjletti, & Eljovich, 2003b). Polymorphisms in the CYP4A11 gene that reduce its catalytic activity by more than 50% are associated with a decrease in the urinary excretion of 20-HETE and the development of salt-sensitive hypertension (Gainer et al., 2005; Gainer et al., 2008; Laffer et al., 2008; Liang et al., 2014; Mayer et al., 2006).

3.2. Vascular remodeling

Remodeling of microvessels occurs in hypertension and involves structural, functional and mechanical changes that result in decreased lumen size, increased media:lumen ratio (M/L) and increased medial thickness (Intengan & Schiffrin, 2001; Schiffrin, 2004). These vascular changes are believed to be important contributors of cardiovascular complications associated with hypertension including end organ damage (Lemarie, Tharaux, & Lehoux, 2010). The mechanisms that contribute to arterial remodeling in hypertension are many and include stimulation of growth and apoptosis, and increased inflammation and fibrosis. Numerous autacoids including bioactive peptides and lipids have been implicated in the process of vascular remodeling. Most prominent among these is Ang II which has been shown to exert the effect of stimulating vasoconstriction, smooth muscle growth, production of inflammatory cytokines and chemokines, and extracellular matrix remodeling; all of which contribute to structural changes and functional remodeling of the arterial wall in hypertension. 20-HETE, which is considered an eicosanoid of the microcirculation (Marji, Wang, & Laniado-Schwartzman, 2002) has effects on the vasculature that resemble those of Ang II and the finding of close relationship between these two autacoids raised the possibility that 20-HETE alone or in concert with Ang II contributes to microvascular remodeling.

To address these possibilities, studies in our laboratory utilized several experimental animal models of hypertension that are all 20-HETE-dependent since either inhibition of 20-HETE biosynthesis or blockade of 20-HETE actions normalize blood pressure and prevents hypertension. In these models, remodeling of the renal microvessels was observed in all of these models. Specifically, in rats treated with -DHT, the hypertension was associated with remodeling of the renal interlobar arteries as evidenced by increases in M/L, medial thickness and collagen deposition. However, this remodeling was independent of blood pressure elevation and could only be prevented by administration of a 20-HETE biosynthesis inhibitor (Ding et al., 2013). Similar results were obtained in the Cyp4a12 transgenic mice in which the expression of Cyp4a12–20-HETE synthase is driven by a tetracyclin-sensitive promoter. In these mice, blood pressure elevation was fully prevented and reversed by ACE inhibitors, AT1R blockers or 20-HETE antagonists. In contrast, vascular dysfunction and remodeling could only be prevented by 20-HETE antagonists, whereas, lisinopril or losartan only lessened remodeling of the renal interlobar arteries (Ding et al., 2013; Garcia et al., 2015). Another study further assessed the contribution of the RAS to 20-HETE-mediated microvascular remodeling by using the androgen-induced hypertension approach in mice lacking endothelial ACE and demonstrated that while remodeling was attenuated compared to wild type mice, it was only prevented by administration of a 20-HETE blocker (Garcia et al., 2015). Hence, 20-HETE's specific contribution to vascular remodeling in hypertension

suggests the existence of cellular mechanisms that are distinct from those activated by Ang II. In further support of this notion, knockdown of the 20-HETE receptor GPR75 prevented remodeling of the renal interlobar arteries in the doxycycline-treated Cyp4a12 transgenic mice (Garcia et al., 2017)

Similarly, a recent study in metabolic syndrome rats showed that 20-HETE antagonists reversed arterial stiffness and systolic hypertension; whereas Losartan did not attenuate arterial stiffness, and only partially reduced systolic blood pressure in these animals (Soler et al., 2018). 20-HETE synthases are not expressed in the large arteries in normal animals. However, in the metabolic syndrome animals, CYP4A and CYP4F are highly expressed in the aorta and carotid arteries. Elevated 20-HETE levels in these animals activated matrix metalloproteinase 12 (MMP12), which in turn, resulted in increased degradation of elastin and increased arterial stiffness (decreased compliance) and systolic hypertension (Soler et al., 2018). It is possible that 20-HETE-dependent regulation of systolic blood pressure is a unique feature of metabolic syndrome (and perhaps obesity and/or obesity-induced type II diabetes) related to high CYP4A and CYP4F expression and resultant high 20-HETE production in large arteries of these animals; ultimately, resulting in increased large artery stiffness - a primary determinant of systolic blood pressure.

3.3. Angiogenesis and arteriogenesis

The bulk of knowledge regarding 20-HETE's role in angiogenesis come from studies of cancer angiogenesis in metastatic cancers. 20-HETE has been shown to promote tumor angiogenesis, growth and metastasis primarily by increasing VEGF (reviewed in (Panigrahy, Kaipainen, Greene, & Huang, 2010).

The role of 20-HETE in angiogenesis in the cardiovascular system is much less clear. A single study showed that 20-HETE induced angiogenesis in a model of hindlimb ischemia in normal animals in vivo (L. Chen et al., 2016). 20-HETE-dependent increase in VEGF synthesis likely underlies this effect (Guo et al., 2009). 20-HETE-VEGF interaction was also documented in skeletal muscle angiogenesis (Amaral et al., 2003). On the other hand, 20-HETE promoted VSMC proliferation and neointima formation (Orozco et al., 2013), cellular responses which we have shown to correlate with impaired arteriogenesis in metabolic syndrome (Hutcheson et al., 2014). Our group recently demonstrated that while in normal animals (Sprague-Dawley rats) relatively low upregulation of CYP4A and CYP4F and induction of comparably low levels of 20-HETE by transient repetitive ischemia (intermittent left anterior descending coronary artery occlusion) was necessary for normal, expected arteriogenic response (coronary collateral growth), high upregulation of CYP4A and CYP4F and induction of very high levels of 20-HETE by transient repetitive ischemia in metabolic syndrome animals (JCR:LA-cp rats) resulted in impairment of arteriogenesis (Joseph et al., 2017). Inhibition of 20-HETE effects by a competitive antagonist, 20-SOLA, completely restored coronary collateral growth in the metabolic syndrome animals, indicating a central role for 20-HETE in the regulation of arteriogenesis. Mechanistically, this effect of 20-HETE was associated with a shift from the low and beneficial, monocyte-mediated inflammatory response in normal animals to a highly exaggerated and neutrophil-based response in metabolic syndrome, which resulted in increased endothelial cell

apoptosis and endothelial dysfunction. The neutrophil-mediated inflammatory response and endothelial dysfunction and apoptosis were likewise completely reversed with 20-SOLA (Joseph et al., 2017). In the aggregate, these results suggest a beneficial role for low, but a detrimental role for high levels of 20-HETE in angiogenesis and arteriogenesis. This distinction may be especially important because basal and ischemia-induced levels of 20-HETE appear to be upregulated in conditions which are risk factors for coronary artery disease, including metabolic syndrome (Joseph et al., 2017), obesity (Kim et al., 2013; Ward, Hodgson, Puddey, Beilin, & Croft, 2006) and diabetes (Issan et al., 2013).

4. 20-HETE function in the heart

4.1. Myocardial Ischemia

Recent studies indicate that 20-HETE may be important in mediating myocardial ischemia-reperfusion injury (IR). A polymorphism in the CYP4A11 gene, the CYP4A isoform which makes 20-HETE in humans, has been associated with increased incidence of myocardial infarction (MI) in human population studies (Fu et al., 2013; Fu et al., 2013). In contrast, the T-T-A haplotype in SNPs rs2269231, rs1126742 and rs9333025 of the CYP4A11 gene was found to be a protective marker against MI in a study of Japanese males but not females (Fu et al., 2012), supporting perhaps the androgen dependence of 20-HETE synthesis observed in the vasculature (Singh & Schwartzman, 2008). Actual 20-HETE levels have not been compared between human patients with IR injury and those without.

We and others have shown that CYP4A and 4F are expressed in the heart and their expression is increased in response to IR in animal models (Gross et al., 2004; Joseph et al., 2017; Nithipatikom et al., 2004). Furthermore, IR has been shown to potently stimulate 20-HETE production in Langendorff heart preparations (Han et al., 2013) and in several studies in animal models of myocardial infarction in vivo in rodents (Gross et al., 2004) and dogs (Nithipatikom et al., 2001; Nithipatikom et al., 2004), as well as in response to transient IR characteristic of stable angina (Joseph et al., 2017), and in response to ischemic preconditioning (Nithipatikom et al., 2006). 20-HETE appears to be produced both in response to ischemia and in response to reperfusion, since the substantial (~2-fold) increase during ischemia is further augmented during reperfusion; however, mechanisms responsible for these increases remain unknown.

Of potential relevance to clinical application, a plethora of inhibitors of 20-HETE biosynthesis (HET0016, DDMS) and 20-HETE actions (5,14 20-HEDE) have been shown to decrease myocardial infarct size in Langendorff heart preparations (Han et al., 2013; Yousif et al., 2009) and in rodent (Gross et al., 2004; Wei et al., 2016) and canine (Nithipatikom et al., 2001; Nithipatikom et al., 2004) hearts in vivo. Similarly, DDMS and 5,14 20-HEDE decreased 20-HETE levels and improved the efficacy of ischemic preconditioning in canine hearts (Nithipatikom et al., 2006). Conversely, administration of 20-HETE increased MI size (Gross et al., 2004; Nithipatikom et al., 2004), further strengthening the causative role for 20-HETE in MI.

One limitation of studies to date is that MI size was evaluated only at short time points (within 2–3) hours after initiation of reperfusion; whereas, MI expansion and immediate

post-MI remodeling often continues at least 24–48 hours post initiation of reperfusion (French & Kramer, 2007). Also, long-term post-MI remodeling, occurring weeks and months post-MI was not investigated. Therefore, the contribution of 20-HETE to final MI size and to intermediate and long-term post-MI remodeling remains unknown. Also of note is the fact that in many of the studies using 20-HETE inhibitors, the inhibitors were administered prior to onset of ischemia or prior to reperfusion (Gross et al., 2004; Wei et al., 2016), therefore; the effect studied is that of a pretreatment vs. of a more clinically relevant treatment, which would require administration at onset of reperfusion or soon after.

The mechanisms that may underlay the effects of 20-HETE on MI are largely unknown. Several well-known mediators of MI and cellular events which are its hallmarks, including increased ROS and cell death have been shown to be significantly attenuated by 20-HETE inhibitors in the setting of IR and MI. 20-HETE has been shown to increase ROS in Langendorff heart preparations, and this was blocked by apocynin suggesting that NAD(P)H oxidases may be involved (Han et al., 2013). However, apocynin is a fairly non-specific NAD(P)H oxidase inhibitor with significant NAD(P)H oxidase-independent anti-oxidant properties. A study in isolated cardiac myocytes did however show that a specific inhibitor of the NAD(P)H oxidase, gp91ds-tat, did inhibit ROS production induced by treatment of the cells with exogenous 20-HETE. In the same study, 20-HETE also induced activation of the L-type Ca^{2+} channel, which was blocked by gp91ds-tat and protein kinase C (PKC) inhibition (Zeng et al., 2010). In IR injury, activation of L-type Ca^{2+} channels may lead to Ca^{2+} overload resulting in contractile dysfunction and necrosis of cardiac myocytes, and thus myocardial stunning and MI expansion. An in vivo study also demonstrated that 20-HETE inhibition increased K^{+} channel activity within the setting of IR (Gross et al., 2004). Decreased K^{+} channel activity has been previously shown to trigger ROS accumulation (Krenz et al., 2002) and Ca^{2+} overload, via membrane depolarization and consequent opening of the L-type Ca^{2+} channels (Downey & Cohen, 2001).

Contribution of 20-HETE to myocyte apoptosis has also been evaluated. A study reported involvement of mitochondrial pathways in 20-HETE-mediated apoptosis in cultured neonatal cardiac myocytes (Bao et al., 2011). Likewise, Inhibition of ω -hydroxylase by DDMS reduced apoptosis in infarcted rat hearts in vivo (Wei et al., 2016), and HET0016 reduced apoptosis in Langendorff-perfused hearts (Han et al., 2013). Ang-II-induced and ROS-mediated mitochondria-dependent apoptosis was attenuated by 20-HETE inhibition (HET0016) (Zhao et al., 2015), suggesting that like in vascular cells, 20-HETE mediates some of the detrimental effects of Ang II in cardiac myocytes. It is however unclear how assessment of apoptosis relates to MI size or post-MI remodeling since necrosis has been established as the predominant mode of myocyte cell death during MI. Moreover, necrosis but not apoptosis stimulates further destruction of the myocardium due to influx of macrophages, their subsequent polarization from phagocytic to the in-inflammatory phenotype in the pro-inflammatory milieu of the MI, and consequent release of further pro-inflammatory cytokines and ROS.

Almost all of these mechanistic studies have been conducted in cultured cardiac myocytes or Langendorff heart preparations, which cannot recapitulate the heart as an integral part of the circulatory system. For example, contribution of inflammation cannot be studied because

recruitment of inflammatory cells to the infarcted heart cannot occur. Because some of the ROS involved in IR injury are derived from inflammatory cells, the full impact of ROS can also not be evaluated. Inflammatory cells also release matrix metalloproteinases and cytokines, all of which play major roles in post-MI remodeling. Furthermore, EPC recruitment is likewise impossible; and thus, the impact and interaction of factors they may contribute cannot be evaluated. Furthermore, 20-HETE has been well-established as a potent pro-inflammatory factor in the vasculature (Hoopes et al., 2015), and inflammation is major contributing factor to IR injury; however, 20-HETE-mediated inflammatory response in IR has not yet been studied. Therefore, the mechanisms by which 20-HETE contributes to IR injury, and by which 20-HETE inhibitors may decrease MI size and post-MI cardiac remodeling in vivo remain largely unexplored, especially in intact animal models in vivo.

4.2. Hypertrophic remodeling and heart failure

Current knowledge regarding the possible role of 20-HETE in hypertrophic cardiac remodeling and cardiac failure is still minimal. CYP4A and 4F are similarly upregulated in patients and animal models of maladaptive cardiac hypertrophy associated with heart failure. CYP4A and 4F were increased in isoproterenol-induced cardiac hypertrophy in rats (Althurwi, Maayah, Elshenawy, & El-Kadi, 2015; Zordoky, Aboutabl, & El-Kadi, 2008). 20-HETE levels were also shown to be elevated in Ang II-induced hypertrophy (Elkhatali, El-Sherbeni, Elshenawy, Abdelhamid, & El-Kadi, 2015). 2015. 289:550–9). Pretreatment with a ω -hydroxylase inhibitor to prevent 20-HETE generation was partially protective against development of cardiac hypertrophy (Alsaad, Zordoky, Tse, & El-Kadi, 2013).

In acute model of doxorubicin-induced cardiotoxicity, a 24h treatment with doxorubicin induced expression of CYP4A1 and A3 and of CYP4F1 and F4 (Zordoky, Anwar-Mohamed, Aboutabl, & El-Kadi, 2010). Although cardiac function was not evaluated, doxorubicin's association with acute and long-term heart failure has been well documented, when administered as a chemotherapy agent (Levis, Binkley, & Shapiro, 2017).

An overall and significant limitation of studies which evaluated the contribution of 20-HETE to cardiac pathophysiology to date is that the vast majority of the studies to date were conducted in normal, healthy animal models. As evident from studies in angiogenesis and arteriogenesis, the effects of 20-HETE may be phenotype-specific, in that CVD risk factors, including obesity, diabetes, hypertension and metabolic syndrome can alter localization of CYP4A expression and increase 20-HETE levels, as well as exacerbate and otherwise modify 20-HETE effects. Only two studies, one from our group, report an association between elevated 20-HETE levels, myocardial ischemia and diabetes in humans (Issan et al., 2013), and reduction in MI size in Langendorff-perfused hearts isolated from diabetic but not from normal rats (Yousif et al., 2009). Both studies indicate a significant effect of diabetes on 20-HETE bioactions and potential effectiveness of 20-HETE inhibitors. Therefore, inclusion of animal models that exhibit these risk factors in future studies may be necessary.

5. 20-HETE in Obesity

Obesity, which is driven primarily by high fat diet and sedentary life style, has a major impact on cardiovascular function. Dietary manipulations, including high fat diet, result in a dramatic induction of CYP4A/F genes, which in animals is mediated through activation of PPAR α whereas in humans via suppression of HNF4 α (Hardwick, 2008; Hardwick et al., 2013). A few studies noted that 20-HETE levels in blood or urine are correlated with body to mass ratio (BMI) in humans (Barden et al., 2007; Issan et al., 2013; Peterson et al., 2016; Tsai et al., 2009; Ward et al., 2006). There are also a few studies demonstrating that 20-HETE levels are increased during the course of high fat feeding and in metabolic syndrome animals (Garbacz et al., 2016; Joseph et al., 2017; Soler et al., 2018; Theken et al., 2012). However, the role of 20-HETE in the pathogenesis of obesity and its associated cardiometabolic conditions including, hypertension, insulin resistance and vascular dysfunction, is unclear.

Studies in our laboratory utilized transgenic mice overexpressing the Cyp4a12–20-HETE synthase and showed that a high-fat diet feeding further increases Cyp4a12 expression and 20-HETE levels in plasma and adipose tissues when compared to corresponding mice fed a regular chow (Ankit et al, unpublished data). The high fat diet-driven hypertension, hyperglycemia and insulin resistance were all prevented and reversed by administration of a 20-HETE antagonist; weight gain was also attenuated by blocking 20-HETE actions (Ankit et al, unpublished data). It appears that the high fat diet, which is known to induce the CYP4A/F genes, leads to increased production of 20-HETE which, in turn, contribute to the hypertension, hyperglycemia and insulin resistance as well as to weight gain in these mice. The question that is yet to be fully answered is how 20-HETE does this. Several studies examined a possible role for 20-HETE in the regulation of insulin signaling and glucose metabolism. Lai et al (Lai et al., 2001) showed that mice overexpressing the human CYP4F2–20-HETE synthase display hyperglycemia with normal insulin levels and functional insulin signaling. Lai et al also showed that 20-HETE has no effect on insulin signaling in vitro using the Bel7402 hepatoma cell line. On the other hand, Li et al (Li et al., 2014) showed that in endothelial cells (HUVECs), 20-HETE impairs insulin-stimulated IRS-1 phosphorylation at Tyr632 while stimulating phosphorylation of IRS-1 at Ser616, which leads to dysfunction of IRS-1 as a docking protein and to disruption of insulin signaling. Another study by Zhang et al (B. Zhang et al., 2016) showed that 20-HETE attenuated glucose-stimulated insulin secretion in mice and in rat insulinoma cell line through suppression of Glut2 expression. In contrast, Tunaru et al (Tunaru et al., 2018) provided substantial evidence that 20-HETE stimulates glucose-stimulated insulin secretion in human and murine islets through binding to the FFAR1 receptor. We have some data to suggest that in high fat diet-fed Cyp4a12-overexpressing mice 20-HETE impairs insulin signaling by inhibiting insulin receptor phosphorylation at Tyr972 and increasing phosphorylation of IRS-1 at Ser307 (Ankit et al, unpublished data). In addition to its effects on insulin signaling, 20-HETE is also adipogenic in vitro (Kim et al., 2013); an effect that may explain our observation that a 20-HETE blocker attenuate weight gain.

The discoveries of GPR75 and FFAR1 (GPR40) as 20-HETE receptor targets open the door to exploration of mechanisms linking 20-HETE to insulin secretion, insulin signaling and

glucose metabolism. Tyrosine phosphorylation is essential for IR and IRS-1 activation whereas serine and threonine phosphorylation of the receptor or its substrate IRS-1 deactivates insulin signaling (Boucher, Kleinridders, & Kahn, 2014). With regard to GPR75 and as depicted in Fig. 2, one may envisage a scenario in which 20-HETE binding leads to a $G_{\alpha q/11}$ -activation of serine/threonine kinases (classical/atypical PKC) that, in turn, phosphorylates the insulin receptor or its substrate on serine and threonine residues. The ability of 20-HETE to increase insulin secretion via binding to FFAR1 is also mediated via $G_{\alpha q/11}$ -driven signaling (Tunaru et al., 2018). Interestingly, Liu et al (B. Liu et al., 2013) reported that isolated human and mouse islets express GPR75 and the chemokine CCL5 increases intracellular calcium levels via activation of GPR75 and suggested CCL5-mediated stimulation of insulin secretion is also a function of GPR75 activation. Binding of CCL5 to GPR75 was not confirmed in these studies (Ignatov, Robert, Gregory-Evans, & Schaller, 2006; B. Liu et al., 2013), however, it is possible that 20-HETE pairing with GPR75 interferes with CCL5-GPR75 binding and modulates CCL5 effect on insulin secretion.

6. Conclusion

20-HETE as a keyword on PubMed yields 795 articles and a wealth of studies attesting to its role in cardiovascular pathophysiology. The review provides a thorough summary of what has been learned regarding 20-HETE bioactions and mechanisms of action in the vascular and cardiac systems, its significant role in the pathophysiology of hypertension and myocardial ischemia-reperfusion injury, and its contribution to obesity-related cardiovascular disease. There are, however, many unanswered questions especially with relation to the relevance of 20-HETE biology in human diseases, its role during development and aging and the impact of physiological and pathological conditions on its biosynthesis and bioactions. The identification of the receptor for 20-HETE along with development of 20-HETE biosynthesis inhibitors and 20-HETE receptor blockers that readily and effectively prevent the action of 20-HETE should provide us with powerful approaches to further understand the pathophysiology of 20-HETE and to design novel therapeutic strategies to combat diseases based on its effects.

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Abbreviation:

20-HETE	20-hydroxy-5,8,11,14-eicosatetraenoic acid
ACE	Angiotensin converting enzyme
Akt	Protein kinase B
Ang II	Angiotensin II
BK_{ca}, Ca²⁺	activated K ⁺ channels
CVD	Cardiovascular Diseases

CYP	Cytochrome P450
CO	Carbon Monoxide
c-Src	Src-type tyrosine kinases
EGFR	Epidermal growth factor receptor
eNOS	endothelial nitric oxide synthase
GPCR	G-protein coupled receptor
GPR75	G-protein coupled receptor 75
IKKβ	Inhibitor of kappa B kinase
NF-κB	Nuclear factor kappa B
MAPK	Mitogen-activated protein kinase
NO	Nitric Oxide
PI3-kinase	Phosphoinositide 3-kinase
ROS	Reactive Oxygen Species
VSMC	Vascular smooth muscle cells

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Fig. 1.

Postulated cellular mechanisms of 20-HETE bioactions in the blood vessel wall. 20-HETE biosynthesis is stimulated by factors such as androgen, high fat, hypoxia/ischemia and vasoactive hormones. 20-HETE binds to its receptor GPR75 and in endothelial cells (EC), triggering a signaling cascade that is initiated by a Gα_{q/11}- and GIT1-mediated c-Src activation and EGFR phosphorylation which is followed by activation of MAPK (ERK1/2) and IKKβ-NF-κB leading to ACE induction, eNOS uncoupling and production of inflammatory cytokines. In vascular smooth muscle cells (VSMC), the activation of Gα_{q/11}- and GIT1 following 20-HETE binding to GPR75 leads to activation of PKC and c-Src, respectively, both kinases phosphorylate the BK channel, inhibiting the BK channel which further sensitize the contractile apparatus. It is also postulated that the Gα_{q/11} activation leads to increase intracellular Ca²⁺ via PLC-IP₃ pathway which further contribute to 20-HETE vasoconstrictor activity. PKC activation following binding of 20-HETE to GPR75 may also be a key to 20-HETE ability to stimulate VSMC migration and proliferation (Garcia & Schwartzman, 2017).

Fig. 2.
Biologic actions of 20-HETE and their contribution to pathophysiology and to the development of vascular and cardiac diseases.