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20-HETE – Hypertension and Beyond

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The Beginning

This review highlights milestones leading to the discovery that 20-hydroxyeicosatetraenoic acid (20-HETE) plays a critical role in the regulation of renal function, vascular tone, and the development of hypertension and cardiovascular disease. Our interest in this pathway emerged 30 years ago. At that time, we were studying mechanisms of pressure natriuresis and factors that reset this response in hypertension. The results indicated that pressure natriuresis is associated with elevations in renal medullary blood flow and interstitial pressure that inhibit sodium transport in the proximal tubule (PT) and thin descending loop of Henle.[1] The pressure-natriuretic response was shifted to higher pressures in spontaneously hypertensive rat (SHR) and Dahl salt-sensitive (S) rats. Resetting of the response in SHR was due to elevated renal vascular resistance. Renal hemodynamics were relatively normal in Dahl S rats, and the blunted natriuretic response was due to elevated sodium transport in the thick ascending loop of Henle (TALH).[2] However, the factors that reset this relationship were unknown.

As presented in Figure 1, we were intrigued with the finding that arachidonic acid (AA) could be metabolized by renal cytochrome P450 (CYP) enzymes to 20-HETE.[3,4] Prior to this, only cyclooxygenase and lipoxygenase enzymes were known to metabolize AA, and the CYP enzymes responsible for ω-hydroxylation of fatty acids were thought to be only expressed in the liver. Iwai et al. then reported that *Cyp4a2* mRNA that produces 20-HETE is differentially expressed in the kidney of Wistar Kyoto and SHR,[5] and Sacerdoti et al. found that synthesis of 20-HETE in the kidney was elevated in SHR.[6] This was followed by a seminal report that treating SHR with $SnCl_2$ reduced renal 20-HETE and attenuated hypertension.[7] However, subsequent studies suggested that the observed fall in blood pressure might be due to induction of the heme oxygenase-carbon monoxide system that can dilate vessels via mechanisms in addition to inhibition of 20-HETE.[8]

Serendipity and the fertile research environment at the Medical College of Wisconsin provided us the opportunity to study the role of CYP metabolites of AA in controlling renal tubular and vascular functions. We were working with Dr. Bettie Sue Masters characterizing the effects of new suicide substrate inhibitors and found that 17-octadecynoic acid (17-ODYA) inhibited formation of 20-HETE.[9] This provided a tool to determine if 20-HETE

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Disclosures

None.

promotes hypertension in SHR by altering vascular tone or the renal handling of sodium. We found that 20-HETE was produced by microsomes prepared from dog renal arterioles and that 20-HETE was a potent constrictor of these vessels.[10] CYP inhibitors reduced myogenic tone in these vessels.[11] In collaboration with David Harder, we found that the vasoconstrictor response to 20-HETE was associated with blockade of the large conductance potassium channel, membrane depolarization, and increase in intracellular calcium concentration.[10,12] Follow-up studies indicated that 20-HETE production was elevated in the kidney and renal microvessels of SHR, which was associated with increased myogenic tone in the afferent arteriole that was normalized by inhibitors of 20-HETE formation.[13] In contrast, 20-HETE synthesis was reduced in the kidney of Dahl S rats.[14] These findings led to the hypothesis (Figure 1) that elevated renal vascular production of 20-HETE contributes to hypertension in SHR by resetting the pressure natriuretic relationship secondary to elevated renal vascular tone, while a deficiency in formation of 20-HETE attenuates pressure natriuresis in Dahl S rats by enhancing tubular sodium reabsorption.

20-HETE Effects on Renal and Vascular Functions

These initial findings triggered a remarkable series of discoveries highlighted in the timeline presented in Figure 2. Elevations in transmural pressure were found to increase formation of 20-HETE in cerebral arteries.[15] Blockade of 20-HETE diminished myogenic tone in renal and cerebral arteries[11,15–18] and autoregulation of renal and cerebral blood flow.[15,17,19] The formation of 20-HETE in blood vessels is increased by angiotensin II (ANG II),[20,21] endothelin,[22] and serotonin.[23] 20-HETE inhibitors attenuated the vasoconstrictor responses to these agonists.[24] 20-HETE was shown to increase vascular tone by activating protein kinase C, mitogen-activated protein kinases, tyrosine kinases, Rho kinase,[24] and promote Ca^{2+} influx by depolarizing the cell membrane secondary to blockade of the large conductance calcium sensitive potassium channel.[10,12] 20-HETE also increases conductance of the L-type calcium channel[25] and activates the transient receptor potential canonical 6 channels.[24] The production of 20-HETE is inhibited by nitric oxide, carbon monoxide, and superoxide that bind to heme in the catalytic site of CYP4A enzymes.[24,26,27] The subsequent fall in 20-HETE levels mediates the cGMP-independent effects of nitric oxide to activate K^+ channels and reduce vascular tone.[27]

Increases in vascular 20-HETE production are associated with endothelial dysfunction in several models of hypertension. These include rats treated with a *Cyp4a2* adenovirus, SHR, androgen-induced hypertensive rats, and *Cyp4a12* transgenic and *Cyp4a14* KO mice in which production of 20-HETE is elevated.[24,28] 20-HETE promotes endothelial dysfunction by uncoupling endothelial nitric oxide synthase and increasing formation of superoxide.[28] More recent studies indicate that increases in vascular 20-HETE also activate the vascular renin-angiotensin system by increasing endothelial expression of angiotensin-converting enzyme.[29]

20-HETE is also a natriuretic agent (Figure 2) that inhibits Na^+/K^+-ATPase activity and sodium transport in the PT,[30,31] as well as in the TALH.[32] Elevations in renal perfusion pressure increase renal 20-HETE, which promotes internalization of the sodium–hydrogen exchanger 3 in the PT and pressure natriuresis.[33,34] 20-HETE inhibits sodium transport in

the TALH by blocking the basolateral Na^+/K^+-ATPase and luminal Na^+-K^+-$2Cl^-$ cotransporter,[32] as well as the luminal 70 pS K^+ channel responsible for K^+ backleak that sustains cotransporter activity.[35]

20-HETE and Hypertension

The advent of the genomic era transformed the field of hypertension. In 1991, J. Rapp argued that it was unacceptable to conclude that a gene plays a causal role in hypertension based on differential gene expression or blood pressure responses to agonists or antagonists in normotensive and hypertensive inbred strains.[36] He proposed that genetic cosegregation analysis was necessary to establish or reject a causal role for a gene in hypertension. Working with Howard Jacob, who had just discovered that simple sequence length polymorphisms were common in rats,[37] we developed polymorphic markers for *Cyp4a2* from published sequences. We found that expression of CYP4A protein and renal formation of 20-HETE was reduced in Dahl S versus Lewis rats and elevated in SHR versus Brown Norway (BN) rats. *Cyp4a2* genotype was associated with hypertension in an F2 cross of Dahl S and Lewis rats fed a high salt diet, but not in a cross of SHR and BN rats fed a normal salt diet.[38,39] Inactivating variants in the coding region of CYP4A11 and 4F2 genes that produce 20-HETE in man were subsequently linked to hypertension, stroke, and cardiovascular disease in human genetic association studies.[40,41]

Expression of CYP4A protein and renal formation of 20-HETE in the kidney, and renal and cerebral vasculature is reduced in Dahl S rats relative to other strains. Dahl S rats do not increase renal epoxyeicosatrienoic acid levels in response to a high salt diet.[41] The impaired pressure natriuretic response in Dahl S rats is mediated by elevated sodium transport in the TALH[42,43] and epithelial sodium channel activity in the cortical collecting duct.[44] Administration of 20-HETE normalized Na^+-K^+-$2Cl^-$ transport in the TALH; however, the elevated sodium transport in the collecting duct was related to decreased formation of epoxyeicosatrienoic acids. Induction of renal 20-HETE and EETs with fibrates[45] or transfer of wild-type *Cyp4a* alleles from Lewis or BN rats increased renal 20-HETE, improved pressure natriuresis, and opposed hypertension and renal injury in consomic and congenic strains[46,47] Vascular 20-HETE production is also reduced in Dahl S rats and this was associated with impaired myogenic and tubuloglomerular feedback responses in the afferent arteriole, autoregulation of renal blood flow and glomerular capillary pressure,[18] increased albumin permeability of the glomerulus,[46] and the development of proteinuria and kidney disease. Transfer of wild-type *Cyp4a* genes in chromosome 5 consomic and congenic Dahl S rats restored the myogenic response of the renal microcirculation and attenuated proteinuria and renal injury.[46,47]

While the original genetic cosegregation analysis excluded mutations in *Cyp4a* genes as a genetic cause of hypertension in SHR, considerable evidence now indicates that vascular 20-HETE serves as a downstream effector to raise blood pressure in various models. In this regard, renal and vascular 20-HETE production is elevated in SHR, and ANG II- and androgen-induced hypertensive rodents.[24,41,48–50] 20-HETE inhibitors attenuate hypertension in male[51] and post-menopausal female SHR[52] and in rats treated with dihydrotestosterone (DHT), which increases 20-HETE production.[49] 20-HETE inhibitors

lower pressure in ANG II- and endothelin-induced hypertensive models.[24,41] Expression of human CYP4A11 and CYP4F2 genes in transgenic mice increases 20-HETE production and blood pressure.[40,41,50] Knockout of *Cyp4a14*, increases expression of *Cyp4a12* and 20-HETE formation and produces salt-resistant hypertension in males secondary to raising testosterone levels.[53,54] These effects are reversed by castration or 20-HETE inhibitors. Increased 20-HETE levels in a conditional *Cyp4a12* transgenic mouse also produced hypertension.[54] The hypertension is associated with elevated vascular reactivity to phenylephrine, increased oxidative stress, and endothelial dysfunction, all of which were reversed by a 20-HETE antagonist.[54]

A summary of the role of 20-HETE in hypertension is presented in Figure 3. Decreased renal formation of 20-HETE in Dahl S rats and patients with inactivating mutations in CYP4A11 and CYP4F2 promote salt-sensitive hypertension. This is associated with impaired myogenic and TGF responses in the afferent arteriole of Dahl S rats, which increases glomerular capillary pressure and renal injury that sustains hypertension. Autoregulation of cerebral blood flow is also impaired in Dahl S rats and this is associated with blood brain barrier leakage.[17] Upregulation of the production of 20-HETE rescued cerebrovascular dysfunction in a *Cyp4a1* transgenic Dahl S rat, suggesting that deficiencies in the formation of 20-HETE may contribute to loss of cognitive function with hypertension and aging.[17]

On the other hand, renal and vascular 20-HETE production are elevated in SHR, ANG II- and androgen-induced hypertensive rodents, and in *Cyp4a14* knockout and *Cyp4a12* and CYP4F2 transgenic mice. These models develop hypertension associated endothelial dysfunction and elevated vascular reactivity. The increase in renal vascular resistance likely shifts the pressure-natriuretic relationship to higher pressures. Increased 20-HETE levels in the kidney may also inhibit sodium reabsorption in the proximal tubule which can help explain the lack of salt-sensitivity of blood pressure in these models. The elevated renal vascular resistance that protects the glomerulus from increases in systemic pressure, likely explains the resistance to proteinuria and renal injury.

20-HETE Inhibitors and Antagonists

Ketoconazole, miconazole and 1-aminobenzotriazole, and other nonspecific agents were the first inhibitors used to reduce 20-HETE in early studies. However, they also inhibit formation of EETs and other CYP enzymes involved in drug metabolism. 17-ODYA was developed as a specific suicide inhibitor of 20-HETE formation,[55] but this compound also inhibits formation of EETs.[9] Dibromo-dodecenyl-methylsulfimide (DDMS) is a more selective 20-HETE inhibitor, but like 17-ODYA, it binds to plasma proteins, is not very soluble or useful for chronic studies.[56]

A major advance was the discovery of formamide derivatives (HET0016, and TS-011) as potent and highly selective inhibitors of 20-HETE formation.[57,58] HET0016 is commercially available and remains the most widely used agent to block synthesis of 20-HETE. TS-011 is a more potent, however, the pharmacokinetic properties of these inhibitors are not favorable, which prevent their development as therapeutic agents. Dr. John Falck synthesized a number

of 20-HETE analogs for structure-activity studies. The screening of these compounds revealed that some competitively blocked the vasoconstrictor actions of 20-HETE, while other analogs were stable agonists.[59,60] This led to the emergence of 20-hydroxyeicosa-6(Z), 15(Z)-dienoic acid (6, 15−20-HEDE) and N-(20-hydroxyeicosa-6(Z), 15(Z)-dienoyl) glycine (6, 15−20-HEDGE) as competitive antagonists of vasoconstrictor actions of 20-HETE.[61] However, these compounds are also highly bound to proteins and have a short half-life, which restricts their use to *in vitro* studies. More recently, Dr. Falck created new water-soluble 20-HETE antagonists, 2,5,8,11,14,17- hexaoxanonadecan-19-yl-20-hydroxyeicosa 6(Z), 15(Z)-dienoate (20-SOLA) and 6(Z),15(Z)-hydroxyeicoas-6,15-dienamido-diencoic acid (AAA).[62,63] These compounds lowered blood pressure when given in drinking water to 20-HETE dependent hypertensive mouse models.

20-HETE in Cardiovascular Diseases

The availability of HET0016 and TS-011 fostered numerous studies on the role of 20-HETE in various diseases, including ischemic and hemorrhagic strokes, acute renal failure, toxemia of pregnancy, hepatorenal syndrome, vascular restenosis, angiogenesis, cardiac hypertrophy, myocardial infarction, renal ischemia-reperfusion injury, and shock.[24,41] 20-HETE is a potent constrictor of cerebral arteries that mimic vasospasm associated with subarachnoid hemorrhage (SAH).[24] Clinical studies indicating that the levels of 20-HETE in cerebrospinal fluid are elevated in patients and experimental animals following SAH.[64] Inhibition of the synthesis of 20-HETE prevented the fall in cerebral blood flow following SAH in rats and reversed delayed vasospasm in rats and dogs.[58,64]

20-HETE also plays a role in ischemic stroke. Levels of 20-HETE are elevated in cerebral tissue and plasma of patients and rats following cerebral ischemia. 20-HETE synthesis inhibitors reduce infarct size and improve neurological outcomes.[64] Mutations in CYP4F2 and CYP4A11 have also been linked to an increased incidence of ischemic stroke in Chinese, Swedish, and Japanese populations.[24,40]

Other studies indicated that the concentration of 20-HETE in coronary venous blood is elevated following cardiac ischemia. Blockade of the synthesis of 20-HETE or administration of a 20-HETE antagonist reduced infarct size and potentiated the cardioprotective effects of ischemic preconditioning.[65] These findings indicate that inhibitors of 20-HETE may be useful to reduce injury following myocardial infarction.

Several reports addressed the role of 20-HETE in renal injury. Blockade of 20-HETE with HET0016 increased, while administration of 20-HETE agonists (5,14-20-HEDGE and 5,14-20-HEDE) reduced bilateral renal ischemic injury.[61] A recent study in Dahl S rats, in which the formation of 20-HETE is reduced, indicated that they are much more susceptible to renal ischemic injury than other strains. Transferring of the CYP4A genes on chromosome 5 from BN rats into the Dahl S genetic background normalized production of 20-HETE and susceptibility to renal injury.[66]

Formation of 20-HETE in carotid arteries was elevated following balloon injury, and chronic administration of HET0016 or other inhibitors of 20-HETE greatly reduced proliferation and

migration of smooth muscle and neointimal formation.[67] This suggests that stents or catheters coated with 20-HETE inhibitors may oppose restenosis. 20-HETE also induces angiogenesis. Inhibition of 20-HETE formation reduced corneal neovascularization produced by several angiogenic growth factors and following implantation of cancer cells.[68] Subsequent studies confirming that 20-HETE inhibitors reduced the vascularization and growth of rat 9L and human U251 glioma tumors in the brain[69] and other cancers has sparked interest in 20-HETE inhibitors as cancer chemotherapy agents.[70]

20-HETE Receptors

The existence of 20-HETE receptors was foretold by the discovery that inactive analogs of 20-HETE are antagonists of its vasoconstrictor actions.[60] Other studies indicating that the vasoconstrictor effects of 20-HETE are phospholipase C/PKC dependent, and its effects on cell migration and proliferation, endothelial dysfunction, and inflammation are c-Src and MAPK pathway dependent, suggest that 20-HETE may act via a G-protein–coupled receptor (GPR).[24] Garcia et al. recently reported that 20-HETE increases vascular tone and promotes endothelial dysfunction by activating a chemokine, RANTES/CCL5, G-protein receptor (GPR75) that signals through the $G\alpha_{q/11}$/PLC/PKC and c-Src/EGFR pathways.[71] It is the first demonstration that a member of this class of eicosanoids acts via a GPR. Activation of this receptor by RANTES was reported to protect the hippocampus from β-amyloid toxicity and to stimulate insulin secretion in pancreatic islet cells.[72] More recently, high concentrations of 20-HETE has been reported to be a potent activator of a the long-chain fatty acid receptor (FFAR1, GPR40) to promote glucose-mediated insulin secretion.[73] However, other studies indicated that 20-HETE inhibits promotes hyperglycemia in CYP4F2 transgenic mice by inhibiting insulin secretion and enhancing glycogenolysis.[74,75] Overall, the new receptor data suggest that other 20-HETE receptors that mediate its multiple actions are yet to be discovered, analogous to multiple receptors that mediate the effects of prostaglandins.

Perspectives

CYPs of the 4A and 4F pathways and 20-HETE play an important role in hypertension and cardiovascular disease. CYP4A or CYP4F enzymes are highly expressed in vascular smooth muscle cells in small arteries and arterioles (<200 μm), the glomerulus, proximal tubule and thick ascending loop of Henle in the kidney, and the heart, liver, prostate, lung and pancreatic islet cells.[24,41] The levels of 20-HETE are altered in numerous disease states. 20-HETE acts as a vasoconstrictor that promotes the myogenic response and autoregulation of RBF and CBF, and inhibits sodium reabsorption in the proximal tubule and thick ascending loop of Henle.[24,41] 20-HETE also serves as a second messenger in the regulation of endothelial dysfunction,[49,50] vascular inflammation,[50] angiogenesis, restenosis, and ischemic injury in the brain, heart, and kidney[24] (Figure 4). Sequence variants in the genes that reduce the production of 20-HETE are associated with hypertension in man.[40,41] GPR75 has recently been identified as the first 20-HETE receptor,[71] and very selective synthesis inhibitors and orally active receptor antagonists are now available (Figure 4). The available data suggests the 20-HETE pathway is a drugable target for the treatment of indications, such as vascular restenosis, cerebral vasospasm and ischemic stroke, myocardial

infarction, and cancer in which the duration of drug administration would be limited. However, given its myriad of actions, it remains to be determined whether 20-HETE inhibitors or antagonists could be safely used to treat chronic diseases, such as hypertension.

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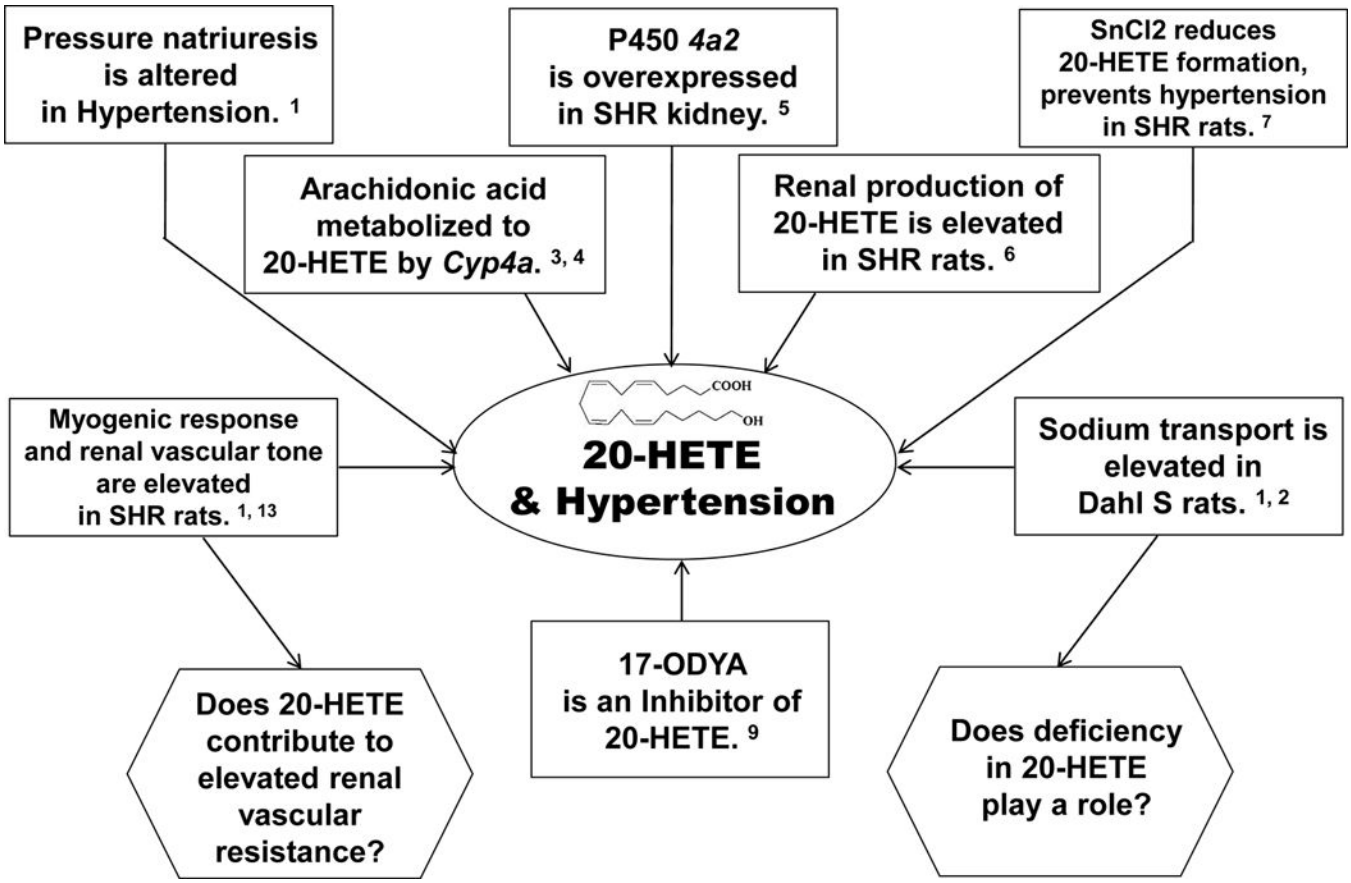

Figure 1.

Initial discoveries implicating 20-HETE in the development of hypertension in the spontaneously hypertensive rat (SHR) and Dahl salt-sensitive (S) rats.

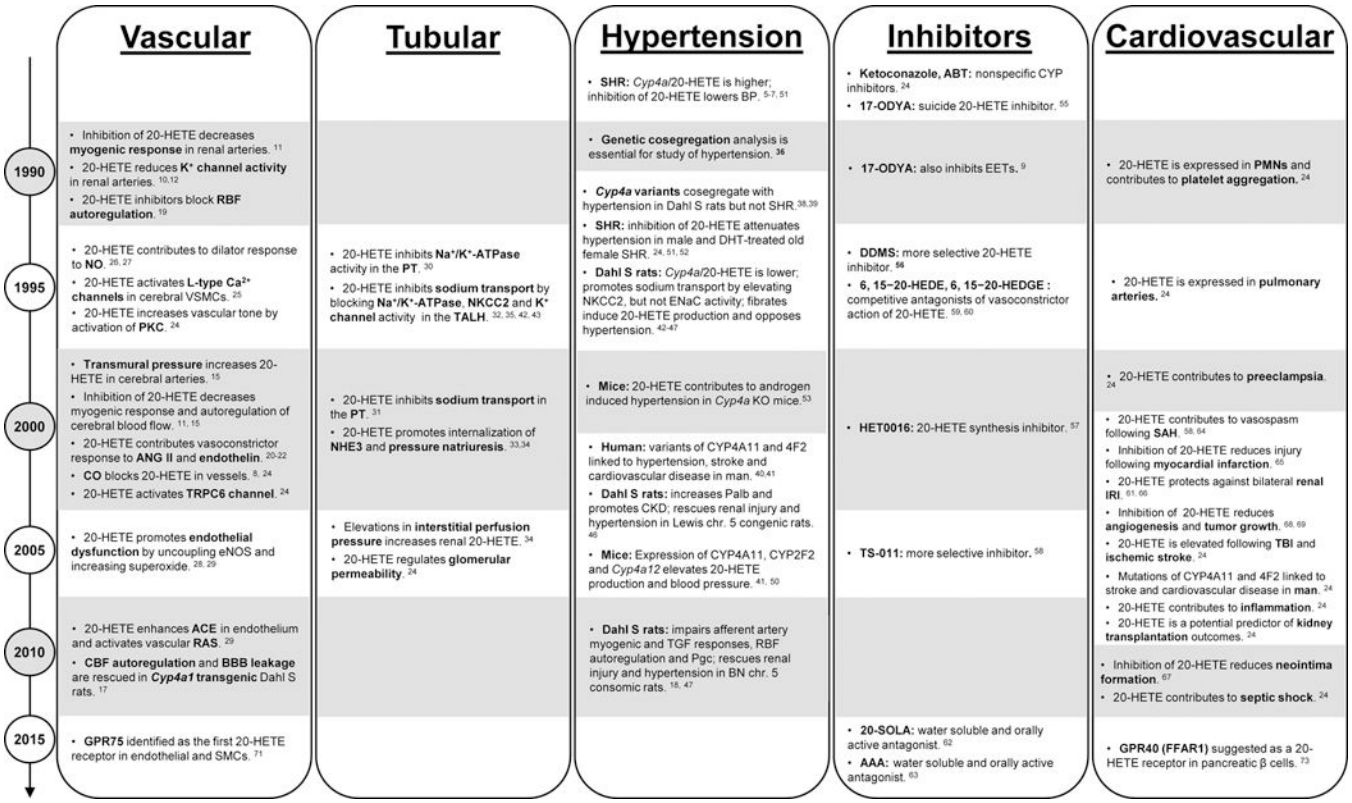

Figure 2.

Timeline highlighting milestones leading to the discovery that 20-HETE plays a critical role in the regulation of renal function, vascular tone, hypertension and cardiovascular diseases

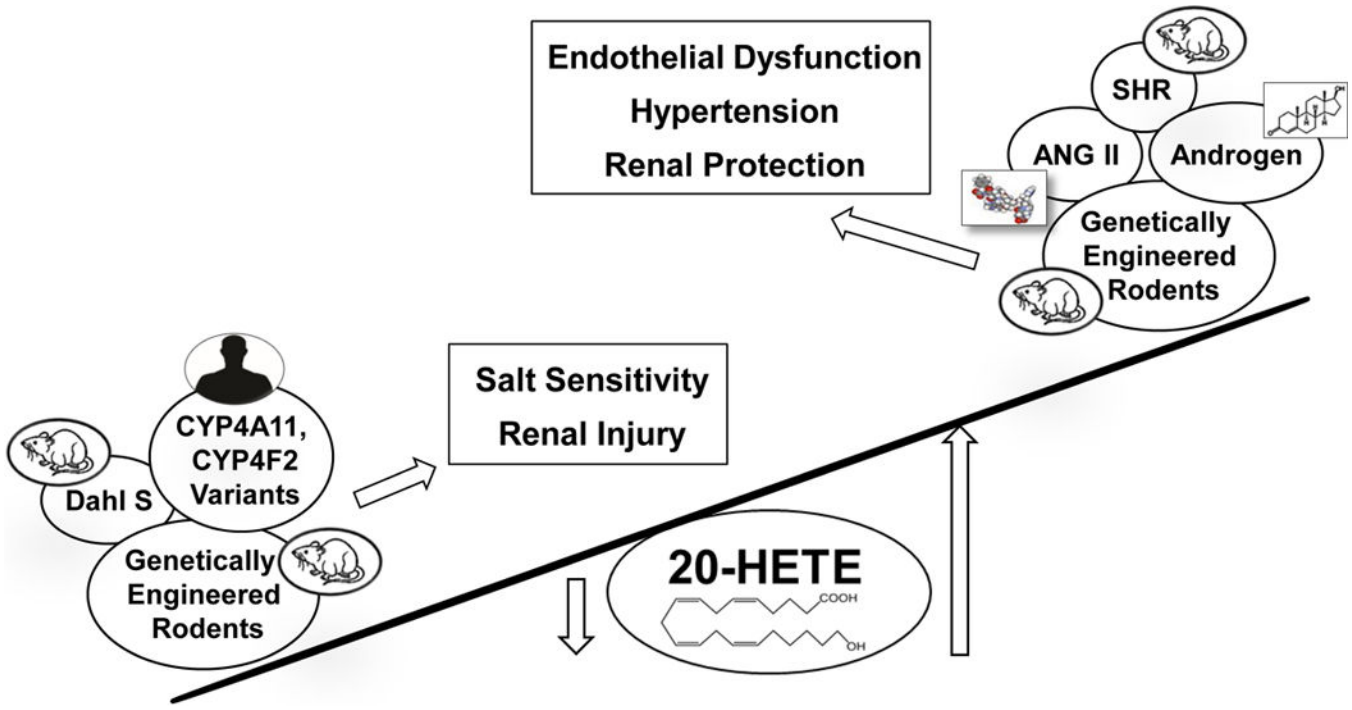

Figure 3.

Role of 20-HETE in genetic and experimental models of hypertension. The renal and vascular production of 20-HETE is altered in various genetic and experimental models of hypertension and in CYP4A11, CYP4F2 transgenic and *Cyp4a* transgenic and KO rodents. In general, elevations in the vascular formation of 20-HETE increase renal and peripheral vascular resistance and produce salt-insensitive forms of hypertension, while decreases in renal 20-HETE increase sodium transport and promote salt-sensitive hypertension.

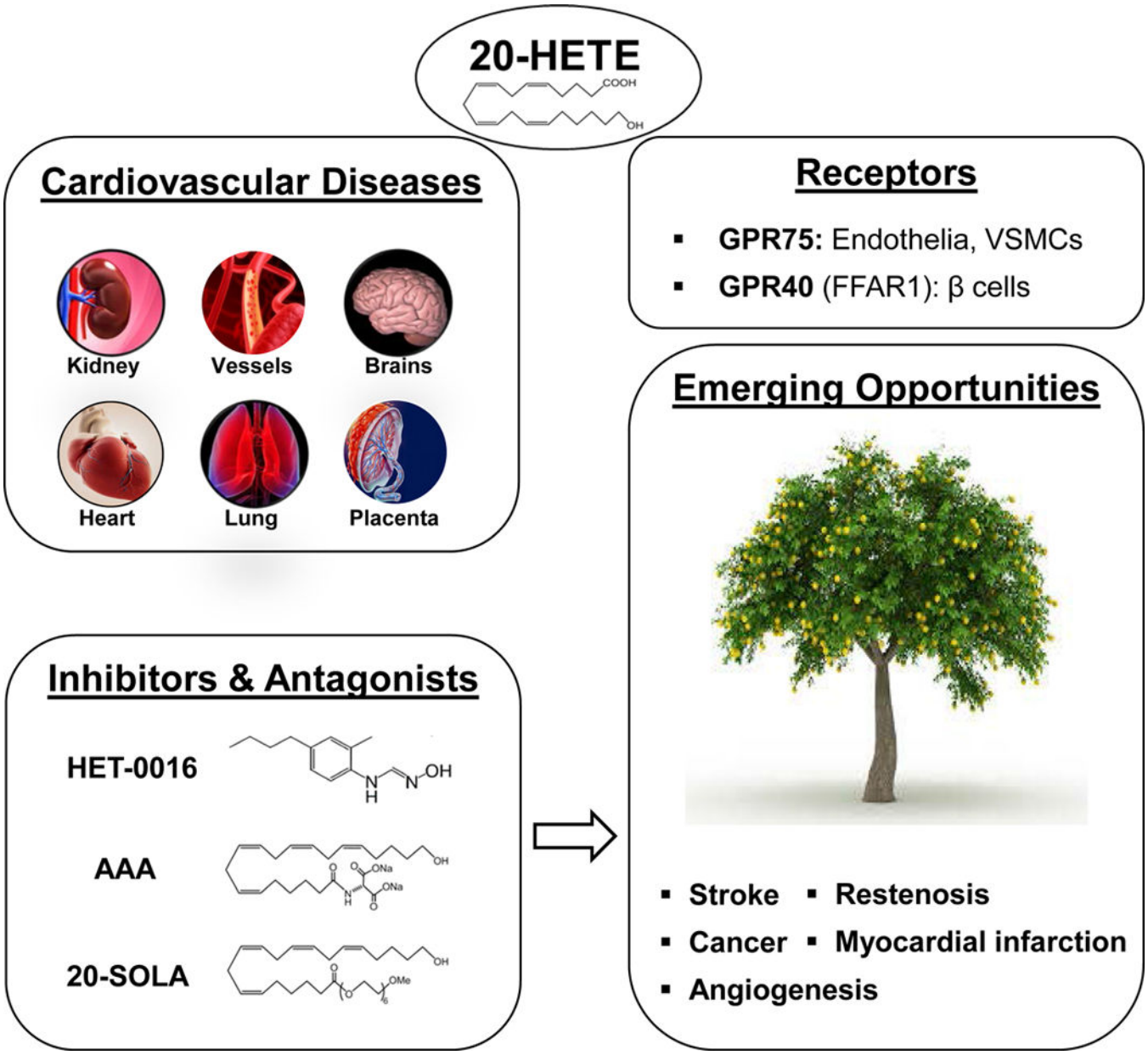

Figure 4.

Emerging opportunities for 20-HETE inhibitors and antagonists in the treatment of cardiovascular disease and cancer.