Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases

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epoxidecatrieic acids; endothelium-derived hyperpolarizing factor; hypotension; nephropathy; inflammation
have been difficult to assess in the past because COX, SEH, and other enzymes can metabolize epoxygenase metabolites. Although renal vascular resistance can either increase or decrease in response to infused regioisomeric EETs, the increase in renal vascular resistance is COX dependent in most of these cases (17, 36, 43, 50). In contrast, the direct application of EETs to renal smooth muscle cells consistently results in dilation and activation of K<sub>Ca</sub> channels (50, 112). This direct application of EETs to the renal microvasculature permits investigation of the vascular smooth muscle cell actions of these metabolites and avoids possible metabolism or degradation that would occur during infusion to an isolated vascular bed. Adventitial administration of 11,12-EET and 14,15-EET dilates the afferent arteriole and this vasodilation is independent of COX activity (50). The corresponding diols, dihydroxyeicosatrienoic acids (DHETEs), lack renal vascular dilator activity (50, 112). DHETEs are generated from EETs by the action of SEH and in a number of vascular systems the diols either have decreased actions or are devoid of activity (43, 87, 105). 5,6-EET is another EDHF candidate because 5,6-EET decreases renal perfusion pressure in the Wistar-Kyoto and spontaneously hypertensive (SHR) rats (80). Interestingly, 11,12-EET and 14,15-EET are the two epoxides of arachidonic acid that most consistently demonstrate vascular smooth muscle cell-relaxing properties and other cardiovascular protective activities (12, 43, 78, 87, 105). Although various regioisomeric EETs are excellent candidates for being an EDHF in the kidney, the afferent arteriolar cellular signaling mechanisms of 11,12-EET are better understood.

The signaling mechanisms utilized by 11,12-EET to elicit dilation of the afferent arteriole clearly establish this epoxide as an EDHF in the kidney. 11,12-EET acts on preglomerular vascular smooth muscle cells to dilate the arteriole (49, 50, 112). In addition, this epoxide activates renal microvascular smooth muscle cell K<sub>Ca</sub> channels (112). The ability of EETs to activate K<sub>Ca</sub> channels is not limited to the renal vasculature or vascular smooth muscle cells (2, 4, 12, 39, 59, 107). 11,12-EET and 14,15-EET have been demonstrated to activate K<sub>Ca</sub> channels in cerebral and coronary vascular smooth muscle cells (4, 12, 39, 59, 107). ADP ribosylation is one intracellular mechanism that has been demonstrated to activate K<sub>Ca</sub> channels in coronary arteries (59). More recently, epoxides have also been shown to hyperpolarize platelets by activating K<sub>Ca</sub> channels and 11,12-EET was the most potent of the regioisomers (58). Renal microvascular activation of K<sub>Ca</sub> channels appears to be mediated by cAMP stimulation of protein kinase A because afferent arteriolar dilation to the sulfonamide analog of 11,12-EET was substantially reduced by protein kinase A inhibition (49). Similarly, 11,12-EET induction of cultured rat aortic smooth muscle cell tissue-type plasminogen activator (t-PA) gene transcription requires activation of Gas, adenyl cyclase, and protein kinase A (79, 94). Taken together, the K<sub>Ca</sub> channel- and protein kinase A-mediated dilator actions of 11,12-EET on afferent arterioles are consistent with the concept that 11,12-EET is an EDHF (Fig. 1).

Further evidence for an epoxygenase metabolite as a renal EDHF has been attained from evaluation of vascular responses to bradykinin and acetylcholine. Bradykinin and acetylcholine are agents that elicit dilation by releasing nitric oxide, prostaglandins, and EDHF (10, 38, 67, 68). Isolated, perfused rat kidney studies were the first to provide evidence that a CYP metabolite contributes to the bradykinin-induced decrease in renal perfusion pressure (37). The development of specific epoxygenase enzyme inhibitors and EET antagonists has enabled investigators to selectively determine the contribution of epoxygenase metabolites to endothelium-dependent vasodilation. Epoxygenase inhibitors can significantly attenuate bradykinin-induced dilation of the afferent arteriole (48). The nitric oxide- and COX-independent rat afferent arteriolar dilation to bradykinin was eliminated by the epoxygenase inhibitor N-methylsulfonyl-6-(2-propargyloxyphenyl) hexamide (MS-PPOH) (48). As further support for involvement of EETs in the preglomerular dilatory response to bradykinin, we demonstrated that renal microvascular EET levels were increased by bradykinin (48). A role for epoxygenase metabolites has also been reported for the afferent arteriolar response in the in vitro perfused hydropnephrotic rat kidney. Wang et al. (98) demonstrated that there were two components to the bradykinin EDHF response of the afferent arteriole. Besides the epoxygenase component, the combination of the K<sup>+</sup> channel inhibitors charybotoxin and apamin blocked another bradykinin-mediated EDHF component that was CYP independent (98). The findings of this study and other studies suggest that endothelial cell charybotoxin- and apamin-sensitive K<sup>+</sup> channels are activated and the resultant hyperpolarization may be transmitted to the underlying smooth muscle layer via myoendothelial gap junctions (88, 98). Last, there is also evidence that glomerular EET production mediates bradykinin dilation of rabbit postglomerular efferent arterioles (86). Although multiple EDHFs appear to contribute to the renal microvascular dilator response to bradykinin, epoxygenase metabolites have been clearly established as an EDHF.

The possible contribution of epoxygenase metabolites to the acetylcholine-mediated afferent arteriolar EDHF response remains unresolved. As has been observed in a number of
vasculatures, the EDHF portion of the afferent arteriolar dilation to acetylcholine is inhibited by the combination of charybdoxin and apamin (97, 98). This acetylcholine EDHF dilatory component was unaltered by the K_\text{Ca} inhibitor tetraethylammonium or the CYP inhibitor 17-octadecynoic acid (97, 98). These studies have led to the postulate that acetylcholine activates endothelial K^+ channels to mediate EDHF vascular smooth muscle cell relaxation independently of CYP450 metabolites. On the other hand, acetylcholine-induced rabbit afferent arteriolar EDHF dilation can be inhibited by the EET antagonist 14,15-epoxyeicosa-5(Z)-enioic acid (96). This study also demonstrated that charybdoxin and apamin eliminate the EDHF component of the rabbit afferent arteriolar dilator response to acetylcholine (96). Thus the exact contribution of EETs to acetylcholine-induced EDHF responses is unresolved and necessitates additional experiments.

In addition to their contribution to endothelium-dependent vasodilators, EETs can also modulate responses to vasoconstrictors. Afferent arteriolar responses to angiotensin are enhanced by CYP inhibition (45). Additionally, rabbit afferent arteriolar dilation to angiotensin has been attributed to angiotensin type 2 (AT_2) receptor activation and EET generation (3). Afferent arteriolar constrictor responses to endothelin-1 (ET-1) are also opposed by epoxygenase metabolites (51). The ability of EETs to counteract the ET-1 constriction of the afferent arteriole did not involve regulation of calcium at the level of the vascular smooth muscle cell because MS-PPOH did not alter the calcium response (51). Similarly, selective epoxygenase inhibitors have also been demonstrated to enhance the afferent arteriolar constriction to elevations in renal perfusion pressure (46). These findings are consistent with the concept that epoxygenase metabolites act as dilators and are a vital component of renal hemodynamic responses.

Another aspect related to EETs biological actions that are garnering attention and excitement are their anti-inflammatory, proliferative, antimigratory, and antithrombotic properties. A connection between inflammatory cytokines and CYP2C enzymes has been clearly established. In regard to the CYP2C enzymes, interleukin-1 (IL-1) suppresses CYP2C11 gene expression in rat hepatocytes via NF-κB binding at the transcription start site (42). IL-6 has also been shown to downregulate CYP2C11 mRNA (73). Intriguingly, cytokines result in the downregulation of endothelial cell CYP2C enzymes and reduce EET-mediated relaxation (54). Node et al. (78, 79) provided the initial evidence that EETs possess anti-inflammato-

CYP2C enzymes enhances NF-κB activity and VCAM-1 expression; however, 11,12-EET was demonstrated to attenuate NF-κB activity in cultured endothelial cells (33). Additionally, the CYP inhibitor sulfaphenazole has recently been shown to enhance endothelium-dependent responses in patients with coronary artery disease (28). CYP inhibition also attenuated the TNF-α-induced increase in cultured endothelial cell mucosal addressin cell adhesion molecule-1 (89). Interestingly, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors have been demonstrated to induce CYP2C, enhance EDHF arterial relaxation, but generate reactive oxygen species (29). Epoxygenase enzyme overexpression has also been shown to protect blood vessels from hypoxia-reoxygenation injury (100). Overall, these studies suggest that CYP2C generation of reactive oxygen species in some instances may be counteracting the beneficial actions of EETs.

The proliferative and antimitratory actions of EETs suggest that EETs are very important for maintaining renal and vascular homeostasis. Growth-mediated actions of EETs are complex and highly suggestive that EETs contribute importantly to vascular injury processes. One consistent finding is that EETs promote endothelial cell proliferation (64, 76, 81, 82). This has been best demonstrated in human endothelial cells that overexpress CYP2C (64, 81, 82). Endothelial proliferation induced by CYP2C9 overexpression or incubation with 11,12-EET activates MAP kinase and upregulates cyclin D1 (82). The endothelial cell response to 11,12-EET also involves phosphatidylinositol 3-kinase (PI3-K) activation of Akt and subsequent inactivation of forkhead box, class O family of transcription factors (81). Interestingly, 14,15-EET activates PI3-K and MAP kinase in renal epithelial cells and promotes proliferation (8, 15, 16). Activation of Src kinase and utilization of the EGF receptor as a scaffold and the resulting MAP kinase activation also appear to be required for 14,15-EET epithelial cell-proliferative responses (14-16). In contrast, the vascular smooth muscle cell responses to EETs have been antimigratory (94). 11,12-EET and CYP2J2 overexpression inhibited rat aorta smooth muscle cell migration in response to serum-derived growth factor or PDGF (94). One confounding finding is that SEH inhibitors attenuate human aortic vascular smooth muscle cell proliferation that is suggestive of an antiproliferative EET action (20). Taken as a whole, these studies imply that EETs are vital components of the renal and vascular response to injury; however, the exact function of epoxygenase metabolites in renal and vascular growth-mediated responses remains to be determined.

Another intriguing action of EETs that would be renal and cardiovascular protective is their profibrolytic activity. Interestingly, the first evidence that EETs possessed antithrombotic actions predated the first description of EET vasodilatory actions (31, 84). All four EET regioisomers were demonstrated to inhibit arachidonic acid-induced aggregation of human platelets (31, 63). More recently, EETs have been demonstrated to induce t-PA expression and hyperpolarize platelets (58, 79). The hyperpolarization of platelets by EETs was associated with inhibition of their adhesion to cultured endothelial cells and induce t-PA expression and hyperpolarize platelets (58, 79). Interestingly, the first evidence that EETs possessed antithrombotic actions predated the first description of EET vasodilatory actions (31, 84). All four EET regioisomers were demonstrated to inhibit arachidonic acid-induced aggregation of human platelets (31, 63). More recently, EETs have been demonstrated to induce t-PA expression and hyperpolarize platelets (58, 79).
Overall, epoxygenase metabolites are an EDHF and are involved in the renal vascular responses to hormones associated with renal and cardiovascular diseases. Moreover, the EET vascular actions beyond those of an EDHF are intriguing. These findings have led investigators to determine the regulation of epoxygenase metabolites during renal and cardiovascular diseases. The possible contribution of the SEH enzyme to the regulation of EET levels and blood pressure regulation is another intriguing area of investigation. Consequently, the renal and cardiovascular protective actions of EETs and SEH are vigorously being explored.

RENAI AND CARDIOVASCULAR PROTECTIVE ACTIONS OF EET AND SEH

It has been recognized for a number of years that CYP metabolites and more specifically renal EETs are involved in renal blood flow regulation and long-term arterial blood pressure control (13, 50, 62, 70, 87). In addition, reports have suggested a significant role for CYP2C and SEH enzymes in the long-term regulation of endothelial function and arterial blood pressure (35, 52, 70, 93, 103). As for the clinical practice, endothelial dysfunction is used as a diagnostic tool and can be a primary target for determining the efficacy of cardiovascular therapy (5, 6, 27, 66). Intriguingly, the protective effects that promote a healthy endothelium coincide with many of the recently described actions of EETs (Fig. 2) (2, 11, 12, 20, 30, 50, 78, 79, 94). Past investigations and recent developments in the areas of CYP2C, epoxygenase metabolites, and epoxide hydrolase that led to their identification as potential renal cardiovascular therapeutic targets will be reviewed.

The regulation and biological actions of epoxygenase metabolites have led to extensive study of these metabolites in blood pressure regulation. As mentioned previously, epoxygenase metabolites can oppose vasoconstrictor actions of the prohypertensive hormones ET-1 and angiotensin (43, 45, 51). In addition to their vascular actions, epoxygenase metabolites affect the flux of ions across epithelial cell membranes, affect cell proliferation, and stimulate hormonal release (13, 40, 43). Regulation of renal EET production has also been intensively studied because the kidney has a relatively high epoxygenase activity (13, 87, 105). CYP2C enzymes and epoxygenase metabolites increase in response to a high-salt diet, and the CYP2C23 appears to be the major epoxygenase in the rat kidney (13, 62, 109). The contribution of CYP metabolites to the pressure-natriuresis response and translocation of tubule sodium transporters has also been established; however, the exact contribution of EETs and the hydroxylase product 20-HETE remains to be determined (22, 44, 106, 108). An increase in EET levels in response to a diet high in sodium would be expected to cause natriuresis because epoxygenase metabolites act to increase renal blood flow and decrease sodium reabsorption (13, 43, 70). As a consequence, hypertension develops in rats that have been administered an epoxygenase inhibitor and fed a high-salt diet (62). Thus regulation of CYP2C enzymes and EETs is important for maintaining body fluid and electrolyte homeostasis and blood pressure in response to a high-salt diet.

Intriguingly, one main finding has been that an inability to increase renal EET levels in response to a high-salt diet has been associated with the elevation in blood pressure observed in salt-sensitive hypertension (62, 65, 69, 109). The elevation in blood pressure and development of hypertension in the Dahl salt-sensitive rats fed a high-salt diet is associated with an inability of these animals to increase renal EET production (61, 62, 87). Lyon hypertensive rats also have a decreased renal epoxygenase activity that contributes to the increase in arterial blood pressure (65, 69). Transgenic rats overexpressing both human renin and angiotensinogen genes (dTGR) develop hypertension and renal failure that are associated with decreased kidney epoxygenase enzymatic activity and CYP2C11, CYP2C23, and CYP2J protein levels (53, 74, 75). Similarly, we have found that an inability to increase renal cortical and vascular CYP2C11 and CYP2C23 protein expression may contribute to salt sensitivity of angiotensin-dependent hypertension (109, 110). Although the CYP2J epoxygenase enzymes are present in the kidney, CYP2J expression does not change in response to a high-salt diet (109). Renal vascular expression of the CYP2J enzyme is decreased in angiotensin-infused rats fed a high-salt diet and could contribute to vascular dysfunction in this type of hypertension (109). Taken together, these studies suggest that increasing epoxide levels in angiotensin-dependent hypertension could lower blood pressure and protect the kidney (Fig. 3).

Even with the promise of epoxygenase metabolites to protect the kidney and vasculature, it has been difficult to assess the possible therapeutic actions of EETs chronically in vivo. Overexpression of epoxygenase enzymes has been used successfully in cell cultures and has only recently been translated to animals (33, 82, 91, 100). Two other approaches have been taken to chronically increase EET levels and determine the epoxide’s renal and vascular beneficial actions. One approach has been to induce renal CYP enzymes with peroxisome proliferator-activated receptor-α (PPARα) activators. PPARα activators, such as fenofibrate and clofibrate, induce CYP2C and CYP4A enzymes and lower triglyceride levels. The second approach has been to inhibit the conversion of the epoxides to their corresponding diols by SEH. As mentioned earlier, SEH

### Renal and Cardiovascular Protective Actions of EETs and SEH

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<td>Anti-Inflammatory Effects</td>
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<tr>
<td>Inhibit Leukocyte Adhesion &amp; Migration</td>
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<td>Inhibit Platelet Aggregation &amp; Adhesion</td>
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**Fig. 2. Renal and cardiovascular protective actions attributed to EETs and soluable epoxide hydrolase (SEH) inhibition. Arachidonic acid can be metabolized to EETs primarily by CYP2C enzymes. EETs are metabolized to bio logically less active or inactive dihydroxyeicosatrienoic acids (DHETEs) by SEH (left). The table on the right represents known vascular actions of EETs or SEH inhibitors and the corresponding references.**
Muller et al. (75) recently demonstrated that PPAR up to 10 days lowered blood pressure and ameliorated renal (103). Similarly, SEH inhibitors administered chronically for period was observed after a single dose of an SEH inhibitor investigated. Blood pressure lowering in SHR over a 24-h for SEH inhibition in blood pressure control has also been giotensin in angiotensin-dependent hypertension (111). A role in inhibition attenuates the afferent arteriolar constriction to an- 

animals (47). Similar to increasing epoxygenase levels, SEH afferent arteriolar reactivity to angiotensin in hypertensive acutely elevating 11,12-EET levels reversed the enhanced 

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investigated. Blood pressure lowering in SHR over a 24-h period was observed after a single dose of an SEH inhibitor (103). Similarly, SEH inhibitors administered chronically for up to 10 days lowered blood pressure and ameliorated renal damage associated with angiotensin hypertension (52, 111). Muller et al. (75) recently demonstrated that PPARα activation with fenofibrate increased renal CYP2C23 activity and pro- 
tected the dTGR hypertensive rat kidney from injury. Fenofi- 
brate treatment increased EET generation and the epoxygenase product of 20-HETE, hydroxy-EETs (HEETs), in dTGR rats 
(75). Decreased blood pressure, inflammation, and renal injury in the dTGR rats treated with fenofibrate are consistent with the 

known EET biological actions; however, the contribution of 

HEETs to renal protection remains unknown. As a whole, these 

studies demonstrate that increasing the levels of EET provides 

protection from angiotensin- and hypertensive-induced renal damage.

The overall potential of epoxides and epoxide hydrolase inhibition to provide beneficial renal and cardiovascular actions in disease states is beginning to be realized. Other studies have demonstrated that elevating 11,12-EET levels or CYP2J overexpression provides blood vessels and the heart protection from hypoxia-reoxygenation injury (23, 91, 100). Addition of 

11,12-EET to transplant preservation solutions can help main- 
tain endothelial function in coronary arteries (101). Although 

the future of epoxygenase metabolites as therapeutic targets 

looks bright, a number of areas still need to be addressed. A 
couple of things that have remained elusive since the first 

biological actions of EETs were described: whether receptors 

for epoxygenase metabolites exist and how intracellular sig- 

naling events are triggered by EETs. In this regard, EET mimetics are already helping investigators to understand the 

structural activity requirements for biological activity (25, 26).

Identification of a binding site or receptor for EETs will 

undoubtedly open up new avenues for investigation. Like 

epoxygenase metabolites, new developments with SEH inhibi- 
tion are on the horizon. The recent findings concerning the 

epoxide hydrolase (EPHX2) gene in humans and localization 
to renal and vascular tissues suggest that experimental findings 
could be translated to patient care (24, 85, 102). Newly devel- 

oped SEH inhibitors have better chemical properties that allow 

these compounds to be administered chronically and orally to 

rodents (55). Fatty acid binding proteins have the ability to 

provide by mentors and the assistance provided by the many collaborators. I 
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Investigator Award (to J. D. Imig).

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Invited Review

EPOXIDES AND HYPERTENSION

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