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Vascular localization of soluble epoxide hydrolase in the human kidney

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Yu, Zhigang, Benjamin B. Davis, Christophe Morisseau, Bruce D. Hammock, Jean L. Olson, Deanna L. Kroetz, and Robert H. Weiss. Vascular localization of soluble epoxide hydrolase in the human kidney. *Am J Physiol Renal Physiol* 286: F720–F726, 2004. First published December 9, 2003; 10.1152/ajprenal.00165.2003.—Epoxyeicosatrienoic acids are cytochrome *P*-450 metabolites of arachidonic acid with multiple biological functions, including the regulation of vascular tone, renal tubular transport, cellular proliferation, and inflammation. Epoxyeicosatrienoic acids are converted by soluble epoxide hydrolase into the corresponding dihydroxyeicosatrienoic acids, and epoxyeicosatrienoic acid hydration is regarded as one mechanism whereby their biological effects are eliminated. Previous animal studies indicate that soluble epoxide hydrolase plays an important role in the regulation of renal eicosanoid levels and systemic blood pressure. To begin to elucidate the mechanism of these effects, we determined the cellular localization of soluble epoxide hydrolase in human kidney by examining biopsies taken from patients with a variety of non-end-stage renal diseases, as well as those without known renal disease. Immunohistochemical staining of acetone-fixed kidney biopsy samples revealed that soluble epoxide hydrolase was preferentially expressed in the renal vasculature with relatively low levels in the surrounding tubules. Expression of soluble epoxide hydrolase was evident in renal arteries of varying diameter and was localized mostly in the smooth muscle layers of the arterial wall. Western blot analysis and functional assays confirmed the expression of soluble epoxide hydrolase in the human kidney. There were no obvious differences in soluble epoxide hydrolase expression between normal and diseased human kidney tissue in the samples examined. Our results indicate that soluble epoxide hydrolase is present in the human kidney, being preferentially expressed in the renal vasculature, and support an essential role for this enzyme in renal hemodynamic regulation and its potential utility as a target for therapeutic intervention.

microvasculature; epoxyeicosatrienoic acids; dihydroxyeicosatrienoic acids

EPOXYEICOSATRIENOIC ACIDS (EETs) are cytochrome *P*-450 metabolites of arachidonic acid (AA) that have potent effects on the renal vasculature and renal tubular transport. Several regioisomeric EETs have been implicated as endothelium-derived hyperpolarizing factors and potently dilate numerous vascular beds through their activation of calcium-dependent K^+ (K_{Ca}) channels in the vascular smooth muscle cell (3). There is also evidence that EETs can prevent vascular inflammation by a mechanism involving inhibition of adhesion molecule expression and that they can modulate the growth of vascular smooth muscle cells in vitro and in vivo (5, 18). In addition, EETs can inhibit Na^+ reabsorption in the renal tubule and mediate the pharmacological action of hormones in the kidney (23).

Soluble epoxide hydrolase (sEH) converts EETs into their corresponding dihydroxyeicosatrienoic acids (DHETs). In general, the DHETs are thought to have reduced biological function relative to the EETs, and hydration of the EETs by sEH represents one mechanism whereby their activity can be attenuated or eliminated. Relative to EETs, DHETs are more hydrophilic and are more likely to be excreted. DHETs also have reduced intracellular protein binding and are incorporated to a lesser degree into the membrane phospholipid pool relative to the corresponding EETs (32, 33). Rapid excretion of the DHETs out of the cell is one possible reason for their reduced biological activity relative to the EETs.

Cellular and animal studies provide evidence that sEH-mediated EET hydration is an important mechanism for the regulation of intracellular EET levels and therefore plays a significant role in vascular inflammation and blood pressure regulation. Chemical inhibitors of sEH have been associated with attenuation of vascular smooth muscle cell proliferation in cultured cells and a reduction of blood pressure in spontaneously hypertensive rats and in an ANG II rat model of hypertension (5, 12, 35). The contribution of sEH to blood pressure regulation is also illustrated by the observation that targeted disruption of the sEH gene lowers systemic blood pressure in male mice (28). The molecular mechanism by which sEH inhibitors modulate blood pressure is not fully understood, although measurement of endogenous eicosanoid levels supports a role for increased intracellular EET levels in their effect.

Relatively little is known about cytochrome *P*-450-catalyzed AA metabolism and the biological effects of the resulting eicosanoids in humans. sEH expression is evident from activity measurements in human liver, kidney, and placental subcellular fractions, but the cellular localization of this enzyme within these organs is not well studied (30, 34). The present studies support the hypothesis that sEH plays an important role in the regulation of both EET levels and systemic blood pressure, by demonstrating localization of sEH in the renal microvasculature. In the small sample size examined, there were no obvious differences in sEH

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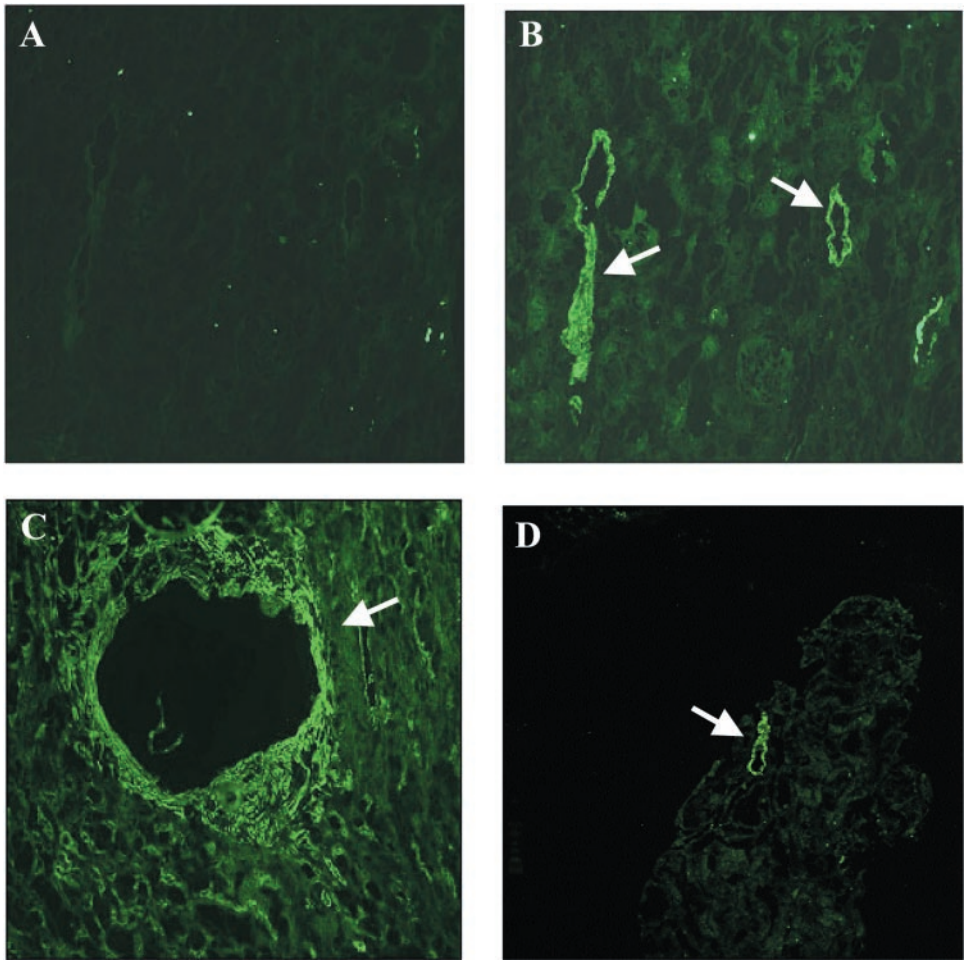

Fig. 1. Low-power magnification (×100) of soluble epoxide hydrolase (sEH) immunoreactivity in sections of normal kidney (*A–C*) and a sample from a focal and segmental glomerulosclerosis (FSGS) patient (*D*). Arrows indicate the specific staining of sEH in the renal vasculature. *A*: negative control lacking primary antibody. *B*: consecutive cross-section from *A* with sEH antibody. *C*: detection of sEH in an arcuate artery in the outer medulla. *D*: immunostaining of sEH in an arteriole in a kidney biopsy sample from a FSGS patient.

localization among disease states. Further study of renal vascular sEH and its regulation may lead to a new class of antihypertensive and anti-inflammatory pharmaceuticals targeting this enzyme.

MATERIALS AND METHODS

Sample collection and handling. Collection of human kidney samples from biopsies and surgical material was approved by the Committee on Human Research at the University of California, Davis and the University of California, San Francisco. A total of 15 human kidney specimens (8 from a variety of diseased kidneys and 7 from normal kidneys) were embedded in optimal cutting temperature-embedding medium (Tissue-Tek, Sakura Finetechnical) and sectioned at −20°C. Kidneys were classified as normal or diseased following microscopic examination by a pathologist (J. L. Olson) for evidence of nephrotic changes, such as mesangial proliferation and glomerular sclerosis.

Immunohistochemistry. Polyclonal anti-sEH antibody against human sEH was raised from rabbits and has been described previously (34). Cryostat sections were fixed in acetone for 15 min at 4°C and blocked with 5% chicken serum (Santa Cruz Biotechnology, Santa Cruz, CA) in PBS for 30 min at room temperature. Diluted sEH antibody (1:200) in PBS solution was immediately applied to the sections and incubated at room temperature for 1 h and then at 4°C overnight. In the negative background control, the sEH antibody was replaced with normal chicken serum. sEH immunolabeling was detected by incubating slides with chicken anti-rabbit serum conjugated with Alexa Fluor 488 (1:200 dilution in BSA-PBS, Molecular Probes, Eugene, OR) for 1 h at room temperature. Slides were then rinsed and placed on coverslips in fluorescence mounting medium (Dako, Carpinteria, CA) and examined using a Nikon TE300 fluorescence microscope equipped with a Bio-Rad MRC 1024 laser-scanning confocal system. In some cases, horseradish peroxidase-labeled secondary antibody was employed and sEH labeling was detected with 3,3′-diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide (Vector Laboratories, Burlingame, CA). Horseradish peroxidase-labeled slides were counterstained with nuclear red (Dako).

Western blot analysis. Kidney tissue was homogenized in buffer containing PBS, 5 mM EDTA, 1 mM PMSF, and 1 mM DTT. Renal S9 fractions containing both cytosolic and membrane subcellular fractions were isolated by centrifuging tissue homogenates at 9,000 *g* for 10 min at 4°C. Samples were then separated on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were incubated with a 1:2,000-fold dilution of human sEH antibody (34) or 1:200-fold dilution of anti-MAP kinase (ERK1 K-23) antibody (Santa Cruz Biotechnology) followed by a 1:15,000-fold dilution of horseradish peroxidase-conjugated anti-rabbit secondary antibody (Sigma, St. Louis, MO). Immunoreactive proteins were visualized using the enhanced chemiluminescence detection kit (Bio-Rad, Hercules, CA).

sEH enzymatic activity. Renal S9 fractions were isolated as described above and used to measure sEH activity using the substrate *trans*-[2-^{3}H]1,3-diphenylpropene oxide (*t*DPPO) as described previously (1). The corresponding activity in a rat kidney S9 fraction was measured for comparison purposes.

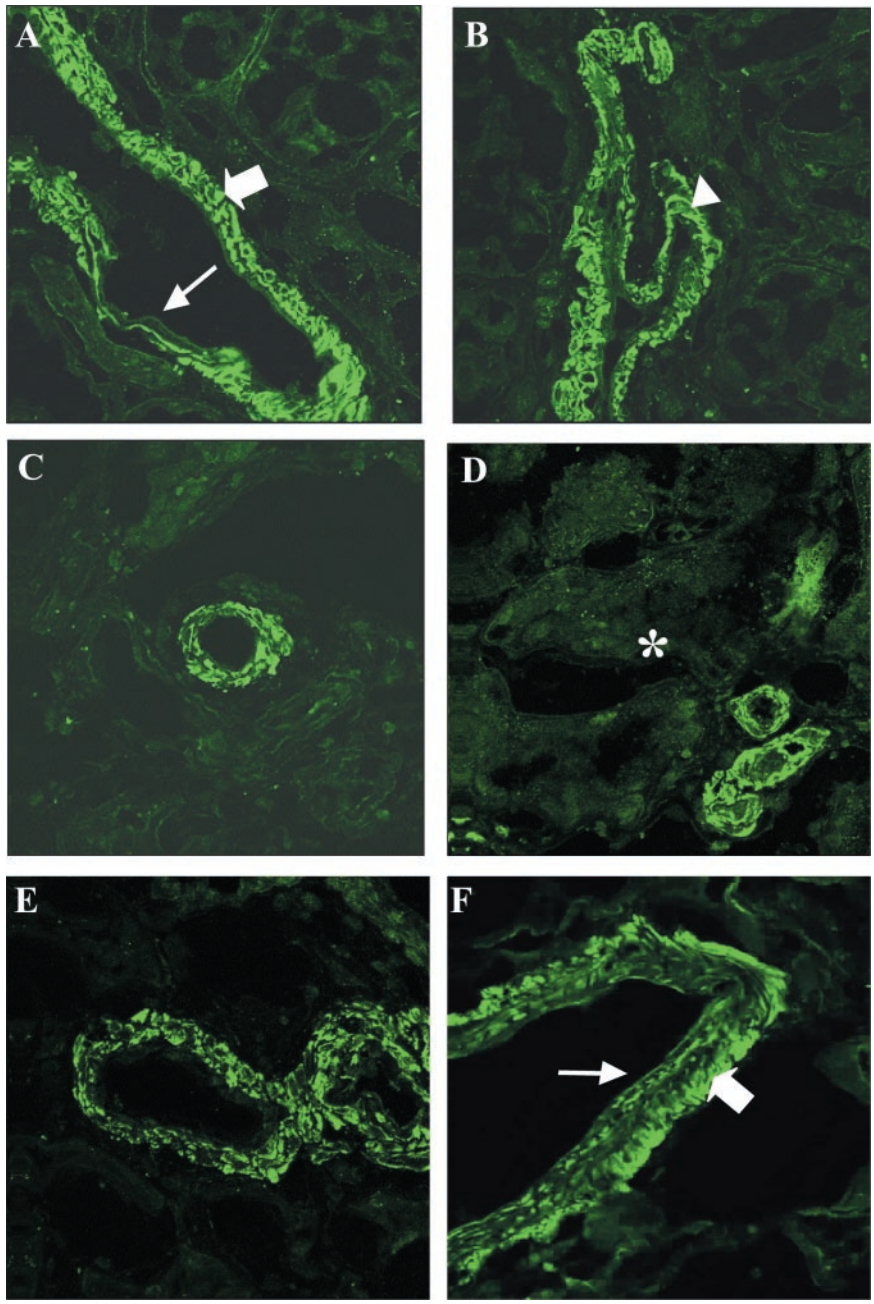

Fig. 2. High-power magnification of sEH immunoreactivity in renal microvessels of varying diameter from a normal human kidney specimen (×400; *A–C*) and from a membrano-proliferative glomerulonephritis (×600; *D*), FSGS (×600; *E*), and a diabetic nephropathy patient (×600; *F*). Strong staining is detected in arteries and arterioles and significantly less in the glomeruli and renal tubules. *A*: renal arcuate artery in the corticomedullary junction. *B* and *C*: renal interlobular arteries and arterioles in the cortex. The arrowhead in *B* indicates the point where an interlobular artery branches into arterioles. *D*: preglomerular arterioles adjacent to a glomerulus (indicated by *). *E* and *F*: arterial staining is in contrast to the weak staining in the surrounding tubule. The arrow in *A* and *F* indicates weak sEH staining in the endothelial layer. The block arrow points to smooth muscle media in the vessel.

RESULTS

Immunohistochemistry. Biopsies were taken during the course of normal clinical practice from patients with systemic lupus erythematosus (SLE) glomerulonephritis, IgA nephropathy, focal and segmental glomerulosclerosis (FSGS), membrano-proliferative glomerulonephritis (MPGN), and minimal change disease (MCD). Normal tissue samples were obtained from cadaveric sources or surgical material. sEH was detected in all 15 human kidney samples (7 normal and 8 diseased). The specificity of the sEH antibody was confirmed by immunofluorescent analysis of sections incubated without primary antibody (Fig. 1*A*). sEH distribution was similar in control and diseased tissue as shown by representative micrographs from normal controls (Figs. 1, *B* and *C*, and 2, *A–C*) and in samples from patients with FSGS (Figs. 1*D* and 2*E*), MPGN (Fig. 2*D*), and diabetic nephropathy (Fig. 2*F*). There was abundant expression of sEH in human kidney, localized largely in the renal vasculature (Figs. 1 and 2). Much weaker immunoreactivity was observed in glomeruli and tubules, and sEH immunostaining in these segments of the nephron was homogeneous (Fig. 2, *C*, *D*, *F*). The sEH protein is localized mostly in the smooth muscle layers of the arterial wall as illustrated in Fig. 2, *A* and *F*. Intense immunoreactivity was detected in arcuate and interlobular arteries and arterioles in the kidney (Figs. 1 and 2). There was abundant sEH expression in the smooth muscle layers of a preglomerular afferent arteriole and an efferent arteriole (Fig. 3). The identity of the afferent arteriole was based on its decreasing diameter toward the glomerulus.

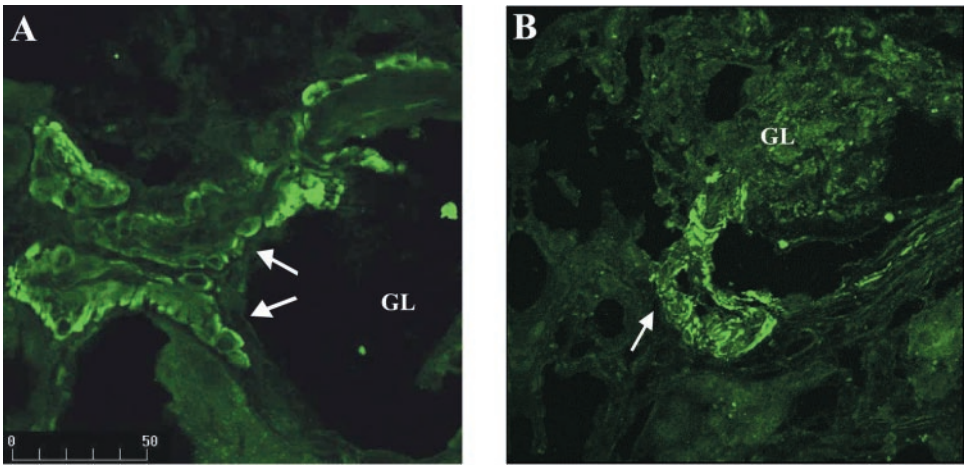

Fig. 3. sEH immunostaining of preglomerular arterioles with adjacent glomerulus (GL) in a section from a normal subject (*A*) and a FSGS patient (*B*). The arrows indicate the preglomerular arterioles. The expression of sEH in the afferent arteriole is much higher than that in the glomerulus and surrounding tubules. Magnification ×600.

Within the glomerulus, a diffuse pattern of sEH expression was observed in mesangial and endothelial cells (Fig. 3).

Immunoperoxidase labeling confirmed the immunofluorescent localization of sEH in the kidney. Strong sEH expression over the entire length of the interlobular and afferent arterioles was also detected by immunoperoxidase staining (Fig. 4). Both efferent and afferent arterioles were positive for sEH expression (Fig. 4*B*).

Western blot analysis. Expression of sEH in human kidney was confirmed by Western blot analysis. Residual biopsy tissue was lysed and separated by SDS-PAGE. sEH-immunoreactive protein was detected as a single major band at a molecular mass of ~60 kDa (Fig. 5). Previous studies in our laboratories showed that the sEH antibody does not cross-react with microsomal epoxide hydrolase or a panel of cytochrome *P*-450 enzymes. There is considerable variability in the expression level of sEH and a control protein, MAP kinase. However, there was sufficient tissue from only three diseased kidneys for analysis of sEH expression relative to MAP kinase, making it difficult to discern whether there are differences in expression between the control and nephrotic kidneys.

sEH enzymatic activity. sEH activity was measured in all human kidney S9 fractions using the substrate *t*DPPO. Hydration of *t*DPPO ranged from 622 to 8,522 pmol·min^{-1}·mg protein^{-1} in diseased kidneys and from 1,217 to 5,492 pmol·min^{-1}·mg protein^{-1} in the controls. Although a large variation in sEH activity is evident in both normal and diseased kidneys (Fig. 6), there is no significant difference in expression between these two groups in the small sample size available for this study (Student's *t*-test, $P = 0.294$). sEH activity in human kidney S9 fractions was comparable to values measured for spontaneously hypertensive rat kidney [2,320 ± 29 pmol·min^{-1}·mg protein^{-1} (35)]. Relatively low sEH activity in two patients is consistent with low-protein abundance as detected by Western blot and evidence of sclerosis in these biopsies.

DISCUSSION

The present study provides the first evidence for localization of sEH in the human kidney. sEH-immunoreactive protein has been localized to the smooth muscle of small- and medium-sized blood vessels in the mouse pulmonary parenchyma (36), but its distribution in the kidney has not been previously characterized in any species. Our finding of sEH localization in renal microvessels is consistent with the observed effect of sEH inhibition on blood pressure (12, 35). sEH catalyzes the hydration of EETs to their corresponding DHETs, a mechanism thought to attenuate or eliminate the biological effects of the EETs. EETs are endogenous constituents of the kidney and are synthesized in renal microvessels, glomeruli, proximal tubules, and thick ascending limb of Henle's loop (2, 4, 13,

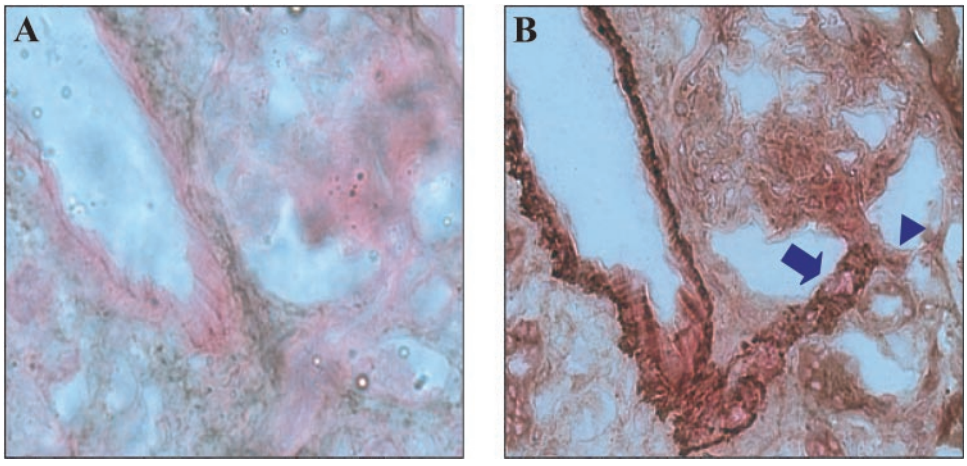

Fig. 4. sEH immunoreactivity in human kidney detected by DAB staining. *A*: negative control lacking primary antibody. *B*: consecutive cross-section from *A* with sEH antibody. Strong immunostaining of sEH (brown) was detected in the renal arteries. sEH expression is evident in both afferent (block arrow) and efferent (arrowhead) arterioles. Nuclei in both slides are shown in red. Magnification ×400.

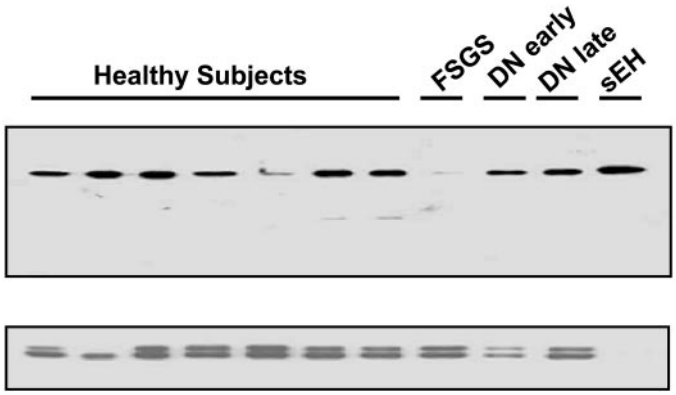

Fig. 5. Expression of sEH in human kidney samples. *Top*: sEH was detected by Western blot analysis in 3 nephrotic and 7 control human kidney samples. *Bottom*: MAP kinase staining was used to compare cellular content between tissue preparations. *Top*: pathological diagnosis of the diseased kidneys. Recombinant sEH protein (sEH) served as a control. DN, diabetic nephropathy.

15). In the artery, EETs are produced predominantly by vascular endothelial cells and migrate to smooth muscle cells to mediate vasodilation (24, 25) and possibly inhibition of vascular smooth muscle cell growth (5). sEH activity in endothelial cell culture is dependent on the species and vascular bed, and in some cases metabolism by sEH is minor relative to β-oxidation pathways (6, 8, 31). The present localization data suggest that EET hydration might be limited in human kidney endothelial cells and that sEH plays a more important role in regulating EET levels in smooth muscle cells. Localization of sEH immunoreactivity largely to vascular smooth muscle cells is consistent with the biological effects of EETs on these cells and the rapid conversion of exogenous EETs to DHETs in smooth muscle cell culture (7).

Our finding that vascular expression of sEH was abundant in afferent arterioles of the kidney, where EETs are likely involved in the regulation of renal blood flow, arterial resistance, and ultimately systemic blood pressure, is consistent with the documented role of sEH in blood pressure regulation in animal models of hypertension and supports a similar role for this enzyme in the human kidney. EETs can serve as downstream messengers for hormones or paracrine factors such as ANG II and bradykinin and regulate the diameter of preglomerular arterioles and glomerular filtration rate (11, 13). EETs also act directly as paracrine factors and communicate signals from the macula densa cells to the vascular smooth muscle cells of preglomerular arterioles and contribute to the tubuloglomerular feedback mechanism (17). The relatively high expression of sEH in afferent arterioles and low abundance in glomerular capillaries suggest that EET levels are tightly regulated for the control of renal hemodynamics. Such placement of sEH also raises the possibility that this enzyme regulates systemic blood pressure and salt balance through renin production by the juxtaglomerular apparatus.

EETs also have anti-inflammatory effects and can inhibit vascular smooth muscle cell proliferation in vitro (5, 18). Endogenous EET levels are increased in the stenosed, endothelially injured coronary artery and in the aorta of hypercholesterolemic animals (20, 21). Our finding of abundant expression of sEH in renal arterial smooth muscle cells suggests that tight control of EET levels in this vascular bed will directly influence the development of atherosclerosis and other vascular injuries, possibly modulating the development of microscopic renal artery stenosis, a common clinical problem leading to both hypertension and renal insufficiency.

EETs are involved in the regulation of renal tubular transport and can inhibit Na^+-K^+-ATPase and a Na^+-K^+-$2Cl^-$ cotransporter in proximal and distal tubules (27, 29). Expression of sEH in renal tubules of the human kidney was low relative to vascular expression. This finding was somewhat surprising, considering the relative contribution of vascular and extravascular structures in renal cell homogenates. sEH was easily detected by Western blot analysis in human kidney S9 fractions, and functional activity was similar to levels in renal homogenates from the spontaneously hypertensive rat, a model with high sEH expression and activity (35). Studies are ongoing to determine whether a similar vascular predominant expression pattern is apparent in the spontaneously hypertensive rat kidney. The low expression of sEH in renal tubules suggests that alternative routes of elimination or incorporation into membrane phospholipids might be more important for tubular EETs. Alternatively, EETs formed in the tubules could be released and taken up into neighboring vascular cells where they could be hydrated into DHETs.

Interindividual variation in sEH activity is expected to influence vascular reactivity, renal tubular transport, and the response to inflammatory stimuli. In this study, we detected a 4.5-fold difference in sEH activity between individual kidney samples in the control group and an almost 14-fold variation in sEH activity in the diseased group. Others showed that interindividual variability in sEH activity is as much as 11-fold in lymphocytes and 539-fold in liver (16, 19). Recently, nonsynonymous single nucleotide polymorphisms in sEH have been associated with functional defects in protein activity (22, 26). In rodents, sEH is inducible by peroxisome proliferators such as the fibric acid hypolipidemic agents and shows gender differences in expression (9, 10). It will be important to consider the implications of interindividual differences in sEH activity caused by either genetic variations or environmental influences in the development of vascular disease and hypertension.

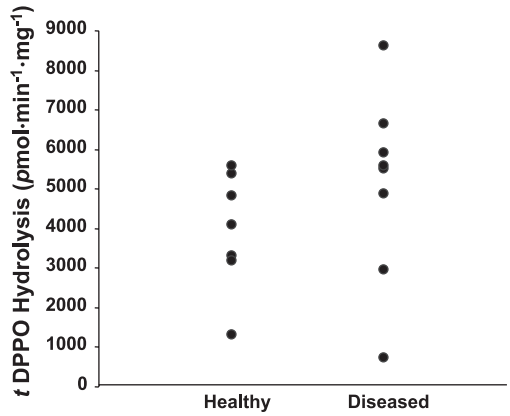

Fig. 6. sEH activity toward *trans*-[2-^{3}H]1,3-diphenylpropene oxide (*t*DPPO) hydration. Activity was measured in S9 fractions from kidney samples (0.1 mg/ml) using [^{3}H]*t*DPPO at a final concentration of 50 μM. Results were normalized to total protein level. There was more variation in activity in the diseased kidneys but no significant difference between control and nephrotic kidneys.

Although the major aim of this study was to localize sEH expression in the human kidney, we were also able to begin to examine the difference in expression between normal and diseased human kidney tissue. EETs are known to have anti-inflammatory and antiproliferative effects in the vasculature and a recent study in a renin and angiotensinogen overexpressed rat model showed that impaired renal hemodynamics and kidney damage were associated with a decrease in EET formation (14). Based on the vascular-specific expression pattern of sEH determined in this study, it is reasonable to hypothesize that sEH tightly regulates EET levels in the vasculature and that its expression could influence the development of glomerulosclerosis and interstitial fibrosis as well as modulate mesangial cell proliferation. In the current study, there were no significant differences in sEH localization, activity, or expression level in kidneys from normal subjects or patients with SLE glomerulonephritis, IgA nephropathy, FSGS, MPGN, and MCD. However, the small sample size in this study does not allow definitive conclusions to be drawn regarding the influence of disease on sEH expression and activity. Further studies with additional samples and well-matched controls are clearly warranted.

In summary, we show that sEH is present in the human kidney in high abundance and is preferentially expressed in the renal vasculature. Abundant sEH expression in smooth muscle cells of renal arteries is consistent with a role for sEH in the regulation of biologically active EET levels and in the regulation of renal vascular tone, salt balance, and inflammation. The possibility that interindividual or disease-modulated variation in sEH expression and function contributes to the pathogenesis of human vascular disease requires further study using a larger sample size. However, our studies suggest that pharmacological inhibition of sEH might be a novel mechanism for modulating vascular EET levels for the treatment of such common human disorders as hypertension and vascular disease.

GRANTS

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REFERENCES

1. **Borhan B, Mebrahtu T, Nazarian S, Kurth MJ, and Hammock BD.** Improved radiolabeled substrates for soluble epoxide hydrolase. *Anal Biochem* 231: 188–200, 1995.
2. **Burns KD, Capdevila J, Wei S, Breyer MD, Homma T, and Harris RC.** Role of cytochrome *P*-450 epoxygenase metabolites in EGF signaling in renal proximal tubule. *Am J Physiol Cell Physiol* 269: C831–C840, 1995.
3. **Campbell WB, Gebremedhin D, Pratt PF, and Harder DR.** Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 78: 415–423, 1996.
4. **Carroll MA, Schwartzman M, Sacerdoti D, and McGiff JC.** Novel renal arachidonate metabolites. *Am J Med Sci* 295: 268–274, 1988.
5. **Davis BB, Thompson DA, Howard LL, Morisseau C, Hammock BD, and Weiss RH.** Inhibitors of soluble epoxide hydrolase attenuate vascular smooth muscle cell proliferation. *Proc Natl Acad Sci USA* 99: 2222–2227, 2002.
6. **Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, Thompson DA, Hammock BD, and Spector AA.** Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. *J Biol Chem* 276: 14867–14874, 2001.
7. **Fang X, Kaduce TL, Weintraub NL, and Spector AA.** Cytochrome P450 metabolites of arachidonic acid: rapid incorporation and hydration of 14,15-epoxyeicosatrienoic acid in arterial smooth muscle cells. *Prostaglandins Leukot Essent Fatty Acids* 57: 367–371, 1997.
8. **Fang X, Weintraub NL, Oltman CL, Stoll LL, Kaduce TL, Harmon S, Dellsperger KC, Morisseau C, Hammock BD, and Spector AA.** Human coronary endothelial cells convert 14,15-EET to a biologically active chain-shortened epoxide. *Am J Physiol Heart Circ Physiol* 283: H2306–H2314, 2002.
9. **Gill SS and Hammock BD.** Distribution and properties of a mammalian soluble epoxide hydrase. *Biochem Pharmacol* 29: 389–395, 1980.
10. **Hammock BD and Ota K.** Differential induction of cytosolic epoxide hydrolase, microsomal epoxide hydrolase, and glutathione *S*-transferase activities. *Toxicol Appl Pharmacol* 71: 254–265, 1983.
11. **Imig JD, Falck JR, Wei S, and Capdevila JH.** Epoxygenase metabolites contribute to nitric oxide-independent afferent arteriolar vasodilation in response to bradykinin. *J Vasc Res* 38: 247–255, 2001.
12. **Imig JD, Zhao X, Capdevila JH, Morisseau C, and Hammock BD.** Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* 39: 690–694, 2002.
13. **Imig JD, Zhao X, Falck JR, Wei S, and Capdevila JH.** Enhanced renal microvascular reactivity to angiotensin II in hypertension is ameliorated by the sulfonimide analog of 11,12-epoxyeicosatrienoic acid. *J Hypertens* 19: 983–992, 2001.
14. **Kaergel E, Muller DN, Honeck H, Theuer J, Shagdarsuren E, Mullally A, Luft FC, and Schunck WH.** P450-dependent arachidonic acid metabolism and angiotensin II-induced renal damage. *Hypertension* 40: 273–279, 2002.
15. **Katoh T, Takahashi K, Capdevila J, Karara A, Falck JR, Jacobson HR, and Badr KF.** Glomerular stereospecific synthesis and hemodynamic actions of 8,9-epoxyeicosatrienoic acid in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 261: F578–F586, 1991.
16. **Mertes I, Fleischmann R, Glatt HR, and Oesch F.** Interindividual variations in the activities of cytosolic and microsomal epoxide hydrolase in human liver. *Carcinogenesis* 6: 219–223, 1985.
17. **Navar LG.** Integrating multiple paracrine regulators of renal microvascular dynamics. *Am J Physiol Renal Physiol* 274: F433–F444, 1998.
18. **Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, and Liao JK.** Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285: 1276–1279, 1999.
19. **Norris KK, DeAngelo TM, and Vesell ES.** Genetic and environmental factors that regulate cytosolic epoxide hydrolase activity in normal human lymphocytes. *J Clin Invest* 84: 1749–1756, 1989.
20. **Pfister SL, Falck JR, and Campbell WB.** Enhanced synthesis of epoxyeicosatrienoic acids by cholesterol-fed rabbit aorta. *Am J Physiol Heart Circ Physiol* 261: H843–H852, 1991.
21. **Pratt PF, Rosolowsky M, and Campbell WB.** Effects of epoxyeicosatrienoic acids on polymorphonuclear leukocyte function. *Life Sci* 70: 2521–2533, 2002.
22. **Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, Bradbury JA, Enayetallah AE, Zeldin DC, and Grant DF.** Polymorphisms in human soluble epoxide hydrolase. *Mol Pharmacol* 64: 482–490, 2003.
23. **Romero MF, Madhun ZT, Hopfer U, and Douglas JG.** An epoxygenase metabolite of arachidonic acid, 5,6-epoxyeicosatrienoic acid, mediates angiotensin-induced natriuresis in proximal tubular epithelium. *Adv Prostaglandin Thromboxane Leukot Res* 21: 205–208, 1991.
24. **Rosolowsky M and Campbell WB.** Role of PGI_2 and epoxyeicosatrienoic acids in relaxation of bovine coronary arteries to arachidonic acid. *Am J Physiol Heart Circ Physiol* 264: H327–H335, 1993.
25. **Rosolowsky M and Campbell WB.** Synthesis of hydroxyeicosatetraenoic (HETEs) and epoxyeicosatrienoic acids (EETs) by cultured bovine coronary artery endothelial cells. *Biochim Biophys Acta* 1299: 267–277, 1996.
26. **Sandberg M, Hassett C, Adman ET, Meijer J, and Omiecinski CJ.** Identification and functional characterization of human soluble epoxide hydrolase genetic polymorphisms. *J Biol Chem* 275: 28873–28881, 2000.
27. **Satoh T, Cohen HT, and Katz AI.** Intracellular signaling in the regulation of renal Na-K-ATPase. II. Role of eicosanoids. *J Clin Invest* 91: 409–415, 1993.
28. **Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, and Gonzalez FJ.** Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* 275: 40504–40510, 2000.

29. **Staudinger R, Escalante B, Schwartzman ML, and Abraham NG.** Effects of epoxyeicosatrienoic acids on ^{86}Rb uptake in renal epithelial cells. *J Cell Physiol* 160: 69–74, 1994.
30. **Thomas H, Schladt L, Doehmer J, Knehr M, and Oesch F.** Rat and human liver cytosolic epoxide hydrolases: evidence for multiple forms at level of protein and mRNA. *Environ Health Perspect* 88: 49–55, 1990.
31. **VanRollins M, Kaduce TL, Knapp HR, and Spector AA.** 14,15-Epoxyeicosatrienoic acid metabolism in endothelial cells. *J Lipid Res* 34: 1931–1942, 1993.
32. **Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P, and Spector AA.** Epoxide hydrolases regulate epoxyeicosatrienoic acid incorporation into coronary endothelial phospholipids. *Am J Physiol Heart Circ Physiol* 277: H2098–H2108, 1999.
33. **Widstrom RL, Norris AW, and Spector AA.** Binding of cytochrome P450 monooxygenase and lipoxygenase pathway products by heart fatty acid-binding protein. *Biochemistry* 40: 1070–1076, 2001.
34. **Wixtrom RN, Silva MH, and Hammock BD.** Cytosolic epoxide hydrolase in human placenta. *Placenta* 9: 559–563, 1988.
35. **Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, and Kroetz DL.** Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res* 87: 992–998, 2000.
36. **Zheng J, Plopper CG, Lakritz J, Storms DH, and Hammock BD.** Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. *Am J Respir Cell Mol Biol* 25: 434–438, 2001.