Angiotensin II receptor blockade or deletion of vascular endothelial ACE does not prevent vascular dysfunction and remodeling in 20-HETE-dependent hypertension

Victor Garcia,1 Gregory Joseph,1 Brian Shkolnik,1 Yan Ding,1 Frank Fan Zhang,1 Katherine Gotlinger,1 John R. Falck,2 Rambabu Dakarapu,2 Jorge H. Capdevila,3 Kenneth E. Bernstein,4 and Michal Laniado Schwartzman1

1Department of Pharmacology, New York Medical College, Valhalla, New York; 2Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas; 3Departments of Medicine and Biochemistry, Vanderbilt University, Nashville, Tennessee; and 4Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, California

Submitted 6 February 2015; accepted in final form 18 April 2015

Garcia V, Joseph G, Shkolnik B, Ding Y, Zhang FF, Gotlinger K, Falck JR, Dakarapu R, Capdevila JH, Bernstein KE, Schwartzman ML. Angiotensin II receptor blockade or deletion of vascular endothelial ACE does not prevent vascular dysfunction and remodeling in 20-HETE-dependent hypertension. Am J Physiol Regul Integr Comp Physiol 309: R71–R78, 2015. First published April 29, 2015; doi:10.1152/ajpregu.00039.2015.—Increased vascular 20-HETE is associated with hypertension and activation of the renin-angiotensin system (RAS) through induction of vascular angiotensin-converting enzyme (ACE) expression. Cyp4a12tg mice, whose Cyp4a12-20-HETE synthase expression is under the control of a tetracycline (doxycycline, DOX) promoter, were used to assess the contribution of ACE/RAS to microvascular remodeling in 20-HETE-dependent hypertension. Treatment of Cyp4a12tg mice with DOX increased systemic blood pressure (SBP; 136 ± 2 vs. 102 ± 1 mmHg; P < 0.05), and this increase was prevented by administration of 20-HEDGE, lisinopril, or losartan. DOX-induced hypertension was associated with microvascular dysfunction and remodeling of preglomerular microvessels, which was prevented by 20-HEDGE, a 20-HETE antagonist, yet only lessened, but not prevented, by lisinopril or losartan. In ACE 3/3 mice, which lack vascular endothelial ACE, administration of 5α-dihydrotestosterone (DHT), a known inducer of 20-HETE production, increased SBP; however, the increase was about 50% of that in wild-type (WT) mice (151 ± 1 vs. 126 ± 1 mmHg). Losartan and 20-HEDGE prevented the DHT-induced increase in SBP in WT and ACE 3/3 mice. DHT treatment increased 20-HETE production and microvascular remodeling in WT and ACE 3/3 mice; however, remodeling was attenuated in the ACE 3/3 mice as opposed to WT mice (15.83 ± 1.11 vs. 22.17 ± 0.92 μm; P < 0.05). 20-HEDGE prevented microvascular remodeling in WT and ACE 3/3 mice, while losartan had no effect on microvascular remodeling in ACE 3/3. Taken together, these results suggest that RAS contributes to 20-HETE-mediated microvascular remodeling in hypertension and that 20-HETE-driven microvascular remodeling independent of blood pressure elevation does not fully rely on ACE activity in the vascular endothelium.

20-HETE; angiotensin II; ACE; vascular remodeling; hypertension; angiotensin-converting enzyme

20-HETE is the ω-hydroxylation product of arachidonic acid metabolism by enzymes of the cytochrome P-450 (CYP) 4A and 4F families. It has been recognized as an eicosanoid of the microcirculation with renal, cerebral, cardiac, and mesenteric arteries having been shown to be rich sources of 20-HETE. Its effects on vascular function are multifaceted and include stimulation of smooth muscle contractility, migration, and proliferation, as well as activation of endothelial cell dysfunction, angiogenesis, and inflammation (1, 2, 4, 21, 34, 36). Such effects could have significant implications with regard to the development of hypertension and its cardiovascular complications. Indeed, numerous studies in experimental models of hypertension have documented a close relationship between increased vascular production of 20-HETE and blood pressure elevation. Models of 20-HETE-driven hypertension also exhibit vascular injury that is exemplified by endothelial and vascular dysfunction (11, 15, 17, 33, 35).

Vascular remodeling is both a product of and contributor to the development of hypertension. This process is promoted by a variety of stimuli, resulting in a melee of structural changes to the vasculature, including collagen synthesis and deposition, reorganization of the extracellular matrix, increased proinflammatory signaling, and altered matrix metalloproteinase activity, rendering vessels stiffer and thicker, thus further exacerbating hypertension (16). We have shown that 20-HETE-driven hypertension is associated with marked remodeling of the renal microvasculature and that the increase in media thickness and media-to-lumen ratio, major hallmarks of vascular remodeling (10), occurred independently of blood pressure elevation and are negated by either a 20-HETE biosynthesis inhibitor or an antagonist of 20-HETE bioactions (6).

The vascular biology of 20-HETE and its implication to the regulation of blood pressure resemble that of ANG II. Both share several characteristics, as each has been shown to promote constrictor stimuli, smooth muscle proliferation and migration, increases in proinflammatory cytokines and chemokines, production of reactive oxygen species, enhanced expression of adhesion molecules, and arterial remodeling in hypertension (4, 21, 25, 34). Our recent study identified 20-HETE as a potent inducer of endothelial angiotensin-converting enzyme (ACE), a critical enzyme involved in the production of the ANG II (3, 6, 30), suggested a close functional crosstalk between these two autacoids in the regulation of blood pressure and vascular function. To this end, experimental models of hypertension that show increased vascular 20-HETE production, such as the spontaneously hypertensive rats (SHR) (7) and androgen-induced hypertension (27, 35, 37), are also ANG II-mediated. Moreover, the blood pressure increase in
models of 20-HETE-driven hypertension is also abrogated by either AT1R blockade or ACE inhibition (30). In cultured endothelial cells, 20-HETE induces ACE expression, and treatment with ACE-specific siRNA prevents 20-HETE-stimulated superoxide production, implicating a role for ACE in the regulation of 20-HETE bioactions (3). Furthermore, administration of a 20-HETE inhibitor to normotensive rats decreased vascular ACE expression (30), suggesting that 20-HETE contributes to the regulation of the vascular renin-angiotensin system under normal physiological conditions. However, the extent to which ACE and ANG II contribute to vascular remodeling in models of 20-HETE-driven hypertension has not been determined. The present study was undertaken to examine the contribution of ACE and ANG II in the development of vascular remodeling in 20-HETE-driven hypertension. Here, we show that 20-HETE-driven microvascular dysfunction and remodeling in hypertension do not fully depend on ACE activity or blood pressure elevation and that 20-HETE serves as a significant mediator of vascular remodeling in hypertension.

MATERIALS AND METHODS

Animal studies. All experimental protocols were approved by the Institutional Animal Care and Use Committee. The generation and phenotypic characterization of the Cyp4a12 transgenic mice in which the expression of the CYP4A12-20-HETE synthase is under the control of an androgen-independent, tetracycline (doxycycline, DOX)-sensitive promoter have been previously described (37). Male and female Cyp4a12tg mice (8–14 wk old) were used in all experiments. DOX (1 mg/ml) was administered in the drinking water for 42 days. The generation of the ACE 3/3 mice (obtained from Dr. Bernstein, Cedars-Sinai) has been previously described (5). In these mice, targeted homologous recombination was used to place the ACE gene under the control of the albumin promoter. The ACE 3/3 mice lack vascular ACE, and ACE expression is restricted to the liver and partial expression in the kidney (5). Male and female ACE 3/3 and age-matched (8–14 wk old) wild-type (WT) mice were used. Placebo or 5α-dihydrotestosterone (DHT)-21-day pellets (5 mg/day, 100 mg/pellet; Innovative Research Group of America, Sarasota, FL) were subcutaneously implanted. In some experiments, mice were administered the 20-HETE antagonists, N-[20-hydroxyecosa-6(Z),15(Z)-di- enoyl]glycine (20-HEDGE; 10 mg·kg⁻¹·day⁻¹ ip in 5% ethanol in saline), lisinopril (10 mg·kg⁻¹·day⁻¹ in drinking water), or losartan (10 mg·kg⁻¹·day⁻¹ in drinking water).

Blood pressure measurements. Systolic blood pressure measurements were taken using the CODA tail-cuff system (Kent Scientific, Torrington, CT), which utilizes volume pressure recording sensor technology. Mice were acclimated to the machine for 1 wk prior to day 0, and blood pressure was monitored throughout the length of the experiment. Values within ±10% of their mean blood pressure measurements were obtained. At the end of the experiment, mice were anesthetized with ketamine (70 mg/kg) and xylazine (70 mg/kg), and laparotomy was performed. Preglomerular arteries were microdissected and collected for Western blot analysis, lipid extraction, and functional studies.

Measurements of 20-HETE. Renal pregglomerular microvessels were isolated and incubated in oxygenated Krebs bicarbonate buffer, pH 7.4, with 1 mM NADPH for 1 h at 37°C with gentle shaking. Deuterated-20-HETE was added as an internal standard, and 20-HETE was extracted and quantified by LC/MS/MS (Applied Biosystems, Foster City, CA), as previously described (37).

Western blot analysis. Renal pregglomerular microvessels were collected and lysed with 1× RIPA (radio-immunoprecipitation assay) buffer (Sigma) containing protease and phosphatase inhibitor cocktails (Roche Applied Sciences, New York, NY). Protein concentrations were determined using the Bradford protein assay (Eppendorf BioPhotometer). Protein samples (20 μg) were loaded onto a 4–20% Mini-PROTEAN TGX precast gel (Bio-Rad, Hercules, CA) with respective loaded EZ-Run Prestained Rec Protein Ladder (Fisher BioReagents, Waltham, MA) markers. SDS-polyacrylamide gels were transferred to Trans-Blot Turbo Mini PVDF membranes (Bio-Rad) followed by blocking buffer (Li-Cor) and subsequent incubation with primary and secondary antibodies. Antibodies included ACE (N-20) (SC-12184; Santa Cruz Biotechnology, Dallas, TX) polyclonal IgG (1:200; Santa Cruz Biotechnology), anti-β-Actin mouse monocular IgG (Sigma), donkey anti-goat IRDye 800CW (1:1,000; Li-Cor), and goat anti-mouse IRDye 800CW (1:10,000). Membrane fluorescence-based immunodetection was conducted using the Li-Cor Odyssey Infrared Imaging System (Li-Cor), and respective band density was quantified using the Odyssey Application software version 3.0.21.

Measurements of vascular function. Renal interlobar arteries were dissected and mounted on a pressurized myograph and equilibrated for 1 h in oxygenated Krebs buffer at 37°C. The operator was blinded to treatments, except for the blood pressure range of the animal. Lumen diameters from normotensive animals were determined at 100 mmHg, and hypertensive animals at 140 mmHg. Measurements of outer diameter (OD) and inner diameter (ID) under passive conditions were used to calculate media thickness [OD − (ID/2)], media-to-lumen ratio [(OD − ID)/ID], and medial cross-sectional area [CSA = (π/4) × (OD² − ID²)].

Measurements of vascular function. Renal interlobar arteries were mounted on wires in the chambers of a multivessel myograph (JP Trading, Aarhus, Denmark) filled with Krebs buffer (37°C) gassed with 95% O₂-5% CO₂. After mounting and 30–60 min of equilibration, the vessels were set to an internal circumference equivalent to 90% of that which they would have in vitro when placed under a transmural pressure of 100 mmHg. Isometric tension was monitored continuously before and after the experimental interventions. A cumulative concentration-response curve to phenylephrine (1 × 10⁻⁸ – 1 × 10⁻⁴ M) was constructed and the maximal relaxation recorded. Wire myograph was used to measure constrictor responses to phenylephrine (10⁻⁸ to 5 × 10⁻⁵ M) and relaxation to Ach (10⁻⁹ to 5 × 10⁻⁵ M) of renal interlobar arteries (~100-μm diameter), as previously described (35).

Statistical analysis. Data are expressed as means ± SE. Significance of difference in mean values was determined using Student’s t-test and one-way ANOVA, followed by the Newman-Keuls post hoc test. P < 0.05 was considered to be significant.

RESULTS

CYP4a12tg mice exhibit 20-HETE- and renin-angiotensin system-dependent hypertension. Administration of DOX to Cyp4a12tg mice increased blood pressure (136 ± 2 mmHg; P < 0.05) compared with vehicle (water)-administered mice (102 ± 1 mmHg). This increase in blood pressure was prevented in mice receiving concurrent treatment of DOX and 20-HEDGE (10 mg·kg⁻¹·day⁻¹) (103 ± 3 mmHg) (Fig. 1). Similarly, mice receiving concurrent treatment of DOX with the ACE inhibitor, lisinopril (10 mg·kg⁻¹·day⁻¹), or the AT1R blocker losartan (10 mg·kg⁻¹·day⁻¹) did not exhibit any blood pressure elevation throughout the 42 days (100 ± 3 and 102 ± 3 mmHg, respectively).
ACE inhibition or AT1R blockade do not prevent vascular dysfunction and remodeling in hypertensive Cyp4a12tg mice. Vascular dysfunction was assessed by measuring the sensitivity to the constrictor activity of phenylephrine and relaxation to ACh in renal interlobar arteries. Arteries from DOX-treated Cyp4a12tg mice displayed increased vascular reactivity, as evidenced by a nine-fold reduction in EC50 to phenylephrine compared with vehicle-treated Cyp4a12tg mice (from 0.73 ± 0.15 to 0.08 ± 0.3 μM) (Fig. 2A). Administration of 20-HEDGE prevented the DOX-induced increase in vascular reactivity (EC50 = 0.89 ± 0.16 μM), while coadministration of losinopril or losartan reduced, but did not prevent, an increase of about six-fold in vascular reactivity (EC50 = 0.51 ± 0.11 μM and 0.45 ± 0.08 μM, respectively). In addition, arteries from DOX-treated Cyp4a12tg mice exhibited impaired relaxation to ACh, compared with arteries from vehicle-treated Cyp4a12tg mice (51% ± 3% vs. 98% ± 1% relaxation). The impaired relaxation response was prevented with cotreatment with 20-HEDGE (89% ± 3% relaxation) (Fig. 2B). Arteries from Cyp4a12tg mice cotreated with DOX and losinopril or losartan displayed an attenuated, yet significant, impairment in relaxation compared with vehicle-treated mice (76% ± 4% vs. 77% ± 1% relaxation for losinopril and losartan, respectively) (Fig. 2B).

Vascular dysfunction in the DOX-treated Cyp4a12tg mice was also exemplified by a significant remodeling of the renal microvasculature. As seen in Fig. 3, media thickness, media-to-lumen ratio, and cross-sectional area were twofold higher (P < 0.05) in arteries from DOX-treated vs. vehicle-treated Cyp4a12tg mice. Moreover, while administration of 20-HEDGE completely prevented the DOX-induced microvascular remodeling, administration of losinopril or losartan only attenuated remodeling by about 40–60% (Fig. 3).

ACE inhibition or AT1R blockade have no effect on vascular 20-HETE production and ACE expression. We also assessed the effect of losinopril and losartan on vascular 20-HETE production and ACE expression. As seen in Fig. 4A, administration of DOX significantly elevated 20-HETE production in preglomerular microvessels (PGMVs) (1.12 ± 0.15 ng/mg protein). Neither 20-HEDGE nor losinopril nor losartan altered the increase in the levels of 20-HETE in PGMVs from DOX-treated Cyp4a12tg mice (Fig. 4A). Measurement of ACE expression in PGMVs revealed that DOX treatment increased ACE protein levels by 4.5 ± 0.8-fold compared with water alone (Fig. 4B). This increase in ACE protein was prevented in mice cotreated with 20-HEDGE, but not in mice cotreated with either losinopril or losartan (Fig. 4B).

Deletion of vascular endothelial ACE attenuates androgen-induced hypertension. To further assess the contribution of 20-HETE-mediated vascular ACE induction to hypertension and vascular dysfunction and remodeling, we used the model of androgen-induced hypertension in ACE 3/3 mice.
mice lack vascular endothelial ACE, exhibit attenuated ACE expression in the kidneys, and display normal ACE and ANG II levels in the plasma (5). As seen in Fig. 5A, ACE protein was undetected in the vasculature (mesenteric arteries and aortae) of either placebo- or DHT-treated ACE 3/3 mice. In contrast, mesenteric arteries and aortae from corresponding WT mice expressed ACE protein, and its expression increased two- to three-fold in response to DHT treatment.

Administration of a 21-day release pellet of DHT, which is a known inducer of 20-HETE production (38), increased production of 20-HETE by three-fold in preglomerular arteries from WT and ACE 3/3 mice alike (Fig. 5B). As was previously shown (9, 37), administration of DHT to WT mice increased systolic blood pressure by 48 ± 4 mmHg by day 14 of treatment and remained elevated throughout the course of 21 days (Fig. 5C). Likewise, DHT increased blood pressure in ACE 3/3 mice; however, the increase, albeit significant, was tamed and amounted to only 25 ± 2 mmHg (Fig. 5C). The blood pressure increases in response to DHT in both WT and ACE 3/3 mice was abrogated by administration of the 20-HETE antagonist, 20-HEDGE, as well as by losartan (Fig. 5C). There were no sex differences in the blood pressure response to DHT in ACE 3/3 mice.

Microvascular remodeling in DHT-treated ACE 3/3 mice is prevented by 20-HEDGE and reduced but not prevented by lisinopril or losartan. Administration of DHT increased remodeling of the renal microvasculature in both WT and ACE 3/3. Media thickness, media-to-lumen ratio, and cross-sectional area increased in PGMV in both WT and ACE 3/3 mice; however, remodeling was attenuated in the ACE 3/3 mice (1.60-fold increase, placebo vs. DHT; P < 0.05) as opposed to the WT mice (2.28-fold increase, placebo vs. DHT; P < 0.05) (Fig. 6). These increases were prevented by coadministration of 20-HEDGE. In contrast, losartan attenuated remodeling in response to DHT in WT mice (2.28-fold increase in DHT and DHT+losartan, respectively; P < 0.05), but not in ACE 3/3 mice (1.60- and 1.56-fold increase in DHT and DHT+losartan, respectively; P < 0.05), suggesting that circu-
lating ANG II does not contribute to this component of vascular remodeling. Importantly, the increase in vascular remodeling in response to DHT in ACE 3/3 was significantly lower (about 60%) than in DHT-treated WT, suggesting the contribution of endothelial ACE to this process (Fig. 6).

DISCUSSION

Previous studies in our laboratory have shown that 20-HETE transcriptionally stimulates endothelial ACE expression via a cellular mechanism that involves the activation of an epidermal growth factor receptor-MAPK- IKK/NF-κB signaling pathway (3). The present study assesses the contribution of endothelial ACE and ANG II to microvascular dysfunction and remodeling in 20-HETE-dependent hypertension using pharmacological probes and genetically modified mice for the Cyp4a12 and ACE genes. The major findings indicate that vascular endothelial ACE contributes to 20-HETE-mediated microvascular remodeling in hypertension and that 20-HETE has effects on microvascular dysfunction/remodeling independent of blood pressure elevation, circulating ANG II, and vascular endothelial ACE induction.

The use of the Cyp4a12 transgenic mice allowed for conditional overexpression of the Cyp4a12, which is the major and perhaps the sole 20-HETE synthase in the mouse (14, 22, 23, 37). Administration of DOX to Cyp4a12tg mice increased systolic blood pressure that was readily abrogated by administration of a 20-HETE antagonist, indicating that the hypertension is driven by increased expression and synthesis of 20-HETE (6, 37). We have previously demonstrated that rats overexpressing the CYP4A2, a major 20-HETE synthesizing enzyme, displayed 20-HETE-driven hypertension that is also associated with increases in components of the renin-angiotensin system (RAS), including ACE activity and levels of ANG II (30). Here, we showed that blood pressure elevation in the DOX-treated Cyp4a12 is RAS-dependent, as both lisinopril and losartan prevented the increase in blood pressure, suggesting that 20-HETE, most likely via induction of ACE expression (3), brings about an activation of the RAS and elevated ANG II that drive the increase in blood pressure. Similar results were obtained in mice lacking endothelial ACE, the ACE 3/3 mice (5), treated with DHT, which have been shown to increase blood pressure in a 20-HETE-dependent manner in
the ACE 3/3 mice was largely attenuated compared with that in WT further underscores the contribution of endothelial/tissue ACE to hypertension. It should be noted that this model is able to maintain sufficient circulating plasma levels of ACE/ANG II and subsequently normal blood pressure (5). However, the lack of tissue ACE in the ACE 3/3 mice may contribute to the tamed hypertensive response to DHT (18).

The demonstration that in both, DOX-treated Cyp4a12tg and the DHT-treated ACE 3/3 mice, administration of a 20-HETE antagonist prevented the increase in blood pressure similarly to lisinopril or losartan suggests that 20-HETE may be an endogenous upstream regulator of the RAS. The finding that in both models, increased expression of the Cyp4a12-20-HETE synthase by either DOX or DHT led to increased levels of 20-HETE that are unaffected by either lisinopril or losartan further enforces this notion. Moreover, in the ACE3/3 mice, administration of DHT resulted in an increase in vascular 20-HETE production that was similar to WT, yet the blood pressure increase due to DHT in ACE 3/3 was about half of that in WT, indicating that tissue ACE is required for the maximum hypertensive response to increases in vascular levels of 20-HETE.

We have previously shown that androgen-driven hypertension is associated with remodeling of the renal microvasculature, a process that is independent of blood pressure elevation and can be prevented by either administration of a 20-HETE biosynthesis inhibitor or a 20-HETE antagonist (6). In the present study, we further showed that the 20-HETE-mediated remodeling in hypertension also occurs in the absence of exogenous androgen stimulus. Hence, the increase in blood pressure in response to DOX in the Cyp4a12tg mouse, which represents a conditional model of androgen-independent 20-HETE-driven hypertension, was also associated with remodeling of the renal microvasculature. All measured parameters for assessing remodeling, including media thickness and media-to-lumen ratio, markedly increased in renal microvessels from CYP4A12tg mice receiving DOX. The remodeling of renal microvessels was fully prevented by the 20-HETE antagonist 20-HEDGE. Interestingly, the changes in vascular remodeling in DOX-treated Cyp4a12tg mice, which were negated by administration of 20-HEDGE, were attenuated but not prevented by lisinopril or losartan. Hence, microvascular remodeling in this model also occurs independent of blood pressure elevation. However, the attenuating effect of lisinopril and losartan suggests that the RAS contributes, in part, to remodeling in 20-HETE-driven hypertension. Findings in the ACE 3/3 mice also support the notion that 20-HETE-driven endothelium-dependent relaxation and remodeling in hypertension ensues independent of blood pressure elevation. Therefore, there is dissociation between blood pressure and end organ injury. Treatment with DHT increased media thickness and media-to-lumen ratio of renal microvessels from both wild-type and ACE 3/3 mice; these increases were prevented by 20-HEDGE. Losartan was used to determine the contribution of AT1R-ANG II to vascular remodeling in this model. Losartan attenuated remodeling in wild-type mice treated with DHT but not in DHT-treated ACE 3/3 mice, suggesting that circulating ANG II does not contribute to vascular remodeling. Importantly, the increase in vascular remodeling in ACE 3/3 was significantly lower (50%) than in wild-type mice, implicating the contribution of endothelial/tissue ACE to this pro-

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

Fig. 6. Deletion of vascular ACE does not prevent DHT-induced 20-HETE-dependent microvascular remodeling of preglomerular arteries in ACE 3/3 mice. Media thickness (A), media-to-lumen ratio (B), and cross-sectional area (CSA) (C) of WT and ACE 3/3 mice treated with placebo, DHT, DHT+20-HEDGE (10 mg·kg⁻¹·day⁻¹), and DHT+losartan (10 mg·kg⁻¹·day⁻¹) for 21 days were measured via pressurized myograph at the end of the experiment (n = 5 or 6). *P < 0.05 vs. placebo-treated mice. #P < 0.05 vs. DHT-treated mice.

rats and mice (14, 26, 28, 29, 35, 37, 38). Hence, administration of DHT to WT mice resulted in an increase in blood pressure that was fully prevented by either 20-HEDGE or losartan. That the RAS plays an important role in androgen-driven hypertension is further substantiated by the finding that the blood pressure increase in response to DHT in the ACE 3/3 mice was only 50% of that in the WT. The fact that losartan was also effective in preventing the blood pressure increase in this model indicates that circulating and/or tissue ANG II are, at least in part, the underlying cause for the hypertension. The finding that the blood pressure increase in response to DHT in
cess. It also should be noted that CYP4a12tg mice receiving DOX displayed elevated ACE expression in renal microvessels. This increase was 20-HETE-dependent, as it was prevented by the 20-HETE antagonist 20-HEDGE. Lisinopril had no effect on DOX-induced ACE expression. Thus, endothelial ACE may be an important contributing factor to the 20-HETE-mediated remodeling in hypertension. The mechanism seems to be, at least in part, independent of its activity and does not rely on ANG II. Additionally, one cannot rule out the involvement of other RAS components, including ACE2 and ANG(1–7) in these studies. Moreover, other autacoids, such as endothelin-1, may play a role in these processes. Interactions between 20-HETE and endothelin-1 have been extensively documented; 20-HETE has been shown to mediate the actions of endothelin-1 in the vasculature, including its effect on smooth muscle proliferation, whereas, endothelin-1 has been shown to stimulate 20-HETE synthesis (13, 19, 20, 31, 32).

Further studies are necessary to determine the cellular mechanisms involved in the onset of vascular remodeling and the distinct contribution of 20-HETE and ACE, as well as other autacoids to this process.

In summary, the present study provides substantial evidence that 20-HETE is a critical mediator of microvascular remodeling in hypertension. It also suggests that induction of ACE, which consequently results in elevation of ANG II levels, is one of the mechanisms by which increases in vascular 20-HETE lead to elevation in blood pressure and alteration in vascular function and structure. The contribution of ACE, whether through its activity or nonenzymatically, to 20-HETE effects in the vasculature needs to be further explored.

Perspectives and Significance

Pathogenic vascular remodeling is a critical feature influencing the development and progression of vascular complications, including atherosclerosis, renal fibrosis, and vascular stenosis (8). Current antihypertensive therapies such as ACE inhibitors or AT1 receptor blockers have been demonstrated to reduce, but never fully reverse, the vascular remodeling associated with hypertension in animal models and clinical observations (12, 24). The present study demonstrates that while blood pressure elevation in models of 20-HETE-driven hypertension is fully prevented and reversed by either ACE inhibitor, AT1R blocker, or 20-HETE antagonist, vascular dysfunction and remodeling can only be prevented by a 20-HETE antagonist: Both losartan and lisinopril attenuate but do not fully prevent the vascular changes in these models. Moreover, using a model of hypertension in which vascular endothelial ACE is absent, we further showed that vascular remodeling only partially relies on activation of the RAS. 20-HETE’s specific contribution to vascular remodeling suggests the presence of cellular mechanisms governing vascular dysfunction and extracellular matrix composition that are distinct from those activated by ANG II. Pharmacological targeting of 20-HETE may serve as a valuable alternative or additive treatment to current antihypertensive therapies, which fall short of treating the vascular complications associated with hypertension.

GRANTS

This study was supported by National Institutes of Health grants HL-034300 (M. L. Schwartzman), DK-038226 (to J. R. Falck), HL-110535 (to K. E. Bernstein), the Robert A. Welch Foundation (I-0011; to J. R. Falck), AHA Founders Affiliate Undergraduate Student Fellowship (1314USF; to B. Shkolnik) and National Heart, Lung, and Blood Institute Diversity Supplement Award HL-34300-26A1S1 (to V. Garcia).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


13. Hercule HC, Oyekan AO. Cytochrome P450 omega-1 hydroxy-lase-derived eicosanoids contribute to endothelin(A) and endothelin(B) receptor-mediated vasoconstriction to endothelin-1 in the rat pregemulor lar arteriole. J Pharmacol Exp Ther 292: 1153–1160, 2000.


