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Soluble epoxide hydrolase-dependent regulation of myogenic response and blood pressure

Dong Sun,[1] Azita J. Cuevas,[3] Katherine Gotlinger,[2] Sung Hee Hwang,[4] Bruce D. Hammock,[4] Michal L. Schwartzman,[2] and An Huang[1]

[1]*Department of Physiology, New York Medical College, Valhalla, New York;* [2]*Department of Pharmacology, New York Medical College, Valhalla, New York;* [3]*New York University School of Medicine, Tuxedo, New York; and* [4]*Department of Entomology or Nematology and University of California Davis Comprehensive Cancer Center, University of California, Davis, California*

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Sun D, Cuevas AJ, Gotlinger K, Hwang SH, Hammock BD, Schwartzman ML, Huang A. Soluble epoxide hydrolase-dependent regulation of myogenic response and blood pressure. *Am J Physiol Heart Circ Physiol* 306: H1146–H1153, 2014. First published February 21, 2014; doi:10.1152/ajpheart.00920.2013.—Epoxyeicosatrienoic acids (EETs) are metabolites of arachidonic acid via cytochrome P450 (CYP)/epoxygenases. EETs possess cardioprotective properties and are catalyzed by soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs) that lack vasoactive property. To date, the role of sEH in the regulation of myogenic response of resistant arteries, a key player in the control of blood pressure, remains unknown. To this end, experiments were conducted on sEH-knockout (KO) mice, wild-type (WT) mice, and endothelial nitric oxide synthase (eNOS)-KO mice treated with *t*-TUCB, a sEH inhibitor, for 4 wk. sEH-KO and *t*-TUCB-treated mice displayed significantly lower blood pressure, associated with significantly increased vascular EETs and ratio of EETs/DHETs. Pressure-diameter relationships were assessed in isolated and cannulated gracilis muscle arterioles. All arterioles constricted in response to increases in transmural pressure from 60 to 140 mmHg. The myogenic constriction was significantly reduced, expressed as an upward shift of pressure-diameter curve, in arterioles of sEH-KO and *t*-TUCB-treated eNOS-KO mice compared with their controls. Removal of the endothelium, or treatment of the vessels with PPOH, an inhibitor of EET synthase, restored the attenuated pressure-induced constriction to the levels similar to those observed in their controls but had no effects on control vessels. No difference was observed in the myogenic index, or in the vascular expression of eNOS, CYP2C29 (EET synthase), and CYP4A (20-HETE synthase) among these groups of mice. In conclusion, the increased EET bioavailability, as a function of deficiency/inhibition of sEH, potentiates vasodilator responses that counteract pressure-induced vasoconstriction to lower blood pressure.

myogenic response; soluble epoxide hydrolase; epoxyeicosatrienoic acids; arterioles; endothelium

THE ISSUE OF HOW THE VASCULAR system maintains basal tone and regulates blood supply and organ perfusion, as well as capillary hydrostatic pressure, has been extensively investigated. Among the above-mentioned functions, the vascular myogenic response is a key contributor. The myogenic response is characterized by the intrinsic ability of small arteries/arterioles to contract in response to increases in transmural pressure. In physiological conditions, the myogenic response functions as an autoregulator that allows the vascular bed to maintain a constant blood flow despite wide changes in arterial pressure. In certain pathological conditions, however, augmented myogenic response initiates an increase in peripheral resistance and consequently increases the risk of hypertension. On the other hand, attenuated myogenic response may exhibit beneficial properties in the regulation of blood pressure, such as an estrogen-dependent reduction of myogenic constriction, resulting in lower blood pressure (17). The nature of the myogenic response of vessels is ultimately dependent on vascular smooth muscle (43); however, vascular endothelium plays an important role in the modulation of the response.

The myogenic response requires a complex mechanotransduction that converts a physical stimulus, such as a change in vascular pressure, into a biological response, which results in changes in vessel diameter. During this process, smooth muscle depolarization and a rise of intercellular calcium are believed to be key steps responsible for the response (16, 51). Since first described by Bayliss (2), research involving cellular mechanisms and signaling cascades responsible for the mediation of the myogenic response has been well investigated. One aspect that has remained consistent throughout this research is the contribution of 20-hydroxyeicosatetraenoic acid (20-HETE), a product of cytochrome P450 (CYP)-catalyzed ω-hydroxylation of arachidonic acid (AA), to the regulation of the myogenic response (26, 41). 20-HETE depolarizes vascular smooth muscle cells and augments myogenic response, which contributes to the pathogenesis of hypertension in a variety of hypertensive models (23, 38, 41, 52, 53). In particular, compared with 20-HETE, fewer studies have investigated the specific effect of epoxyeicosatrianoic acids (EETs; also metabolites of AA through CYP-epoxygenases) on the regulation of myogenic response via a hyperpolarizing mechanism. A stable arteriolar diameter, in response to the distension of the vessel wall, is determined by the balance between the depolarization and hyperpolarization of the smooth muscle cells. In this context, not only ω-hydroxylases (20-HETE synthases), but also epoxygenases (EET synthases) merit consideration as pressure targets of vessels that depolarize/constrict to 20-HETE and hyperpolarize/dilate to EETs (42). In accordance with this concept, altered levels of renal EETs and 20-HETE have been demonstrated in various studies to be associated with changes in blood pressure (1, 54, 55). In vascular endothelial cells, epoxygenase synthesizes EETs that initiate vasodilation (3, 34, 47, 49). Also, the endothelium expresses soluble epoxide hydrolase (sEH) that rapidly hydrolyzes EETs to their corresponding diols (dihydroxyeicosatrienoic acids, DHETs) (6, 8), which, in general, lack vasodilation capacity (25, 28). As such,

Address for reprint requests and other correspondence: A. Huang, Dept. of Physiology, New York Medical College, Valhalla, NY 10595 (e-mail: an_huang@nymc.edu).

both the potentiation of vascular EET synthesis and the reduction of EET hydrolysis would increase EET bioavailability, which in turn would result in the reduction of vascular tone. In this regard, both epoxygenase and sEH can serve as therapeutic targets for hypertension. Given that the myogenic response is an important local regulator responsible for the control of peripheral resistance and that the roles of EETs in modulating the myogenic response are still unknown, we tested the hypothesis that potentiating endothelial EETs, as a function of diminishing their degradation, attenuates myogenic constriction and consequently lowers blood pressure. By using the sEH knockout (KO) mouse which is nulled for the gene encoding the sEH enzyme, and a pharmacological inhibitor of the sEH enzyme, we provide evidence of an endothelium-dependent, EET-mediated modulation of pressure-induced constriction in both normotensive and hypertensive mice.

MATERIALS AND METHODS

Animals. Twelve-week-old male Ephx2$^{-/-}$ (sEH-KO) and Ephx2$^{+/+}$ (wild type; WT) mice were used. Cryorecovered heterozygotes (Ephx2$^{+/-}$, B6.129X-Ephx2tm1Gonz/J) and WT (Ephx2$^{+/+}$) mice were received from Jackson laboratory (Bar Harbor, ME) and the homozygous (Ephx2$^{-/-}$) of sEH-KO mice were developed in the Department of Comparative Medicine, New York Medical College. Eight-week-old male endothelial nitric oxide synthase knockout (eNOS-KO) mice were purchased from the Jackson Laboratory. One group of eNOS-KO mice received *t*-TUCB (a sEH inhibitor, 1 mg/kg per day), via oral gavage, for 4 wk. C57BL/6J mice were used as WT controls for both the sEH-KO and eNOS-KO mice.

All protocols were approved by the institutional care and use committee of New York Medical College and conformed to the guidelines of the National Institutes of Health and the American Physiological Society for the use and care of laboratory animals.

Genotype. Primers for sEH were synthesized according to the information provided by Jackson Laboratory. The standard PCR was performed using an EZ Tissue PCR Genotyping Kit (EZ BioResearch).

Measurement of blood pressure. Mice were anesthetized by inhalation of Isothesia (isoflurane). Blood pressure was recorded using a carotid artery catheterization at a controlled heart rate of ~450 beats/min by adjusting the depth of anesthesia.

Myogenic response. The microcirculation of skeletal muscle is responsible for the sizable fraction of peripheral resistance. To this end, both sides of the gracilis muscle were dissected, and second-order arterioles were isolated and cannulated in a vessel chamber perfused with physiological salt solution (PSS). Isolated arterioles were equilibrated, under 80 mmHg intraluminal pressure and zero intraluminal flow condition, for 1 h. During this time, all vessels developed spontaneous tone. The intraluminal pressure was then lowered to 20 mmHg and subsequently increased to 140 mmHg in 20-mmHg steps. Each pressure step was maintained for 5–10 min to allow the vessels to reach a stable condition. Changes in diameter of the arterioles in response to the incremental intraluminal pressure were recorded. The pressure-diameter relationship of WT and sEH-KO mice was assessed in the control condition and also after removal of the endothelium. Removal of the endothelium was accomplished by injecting 1 ml air through the lumen of the vessels at 40 mmHg of injection pressure. The vessel was then perfused with PSS (10 μl/min) for 30 min and reequilibrated with zero intraluminal flow for 1 h before the pressure diameter relationship was reassessed. The efficiency of the endothelial denudation was tested by evaluation of endothelium-dependent and -independent vasodilator responses to acetylcholine (ACh, 5×10^{-8} mol/l) and sodium nitroprusside (SNP, 10^{-7} mol/l) before and after the procedure of endothelial removal. In separate experiments, the pressure-diameter relationship was recorded before and after incubation of vessels with N^{ω}-nitro-L-arginine methyl ester (L-NAME; 3×10^{-4} mol/l; an inhibitor of nitric oxide synthases) for 40 min, to evaluate effects of endothelial nitric oxide (NO) on the response. Additionally, the pressure-induced myogenic constriction in gracilis muscle arterioles of eNOS-KO mice, treated with and without *t*-TUCB, was also assessed in the control and after exposure of vessels to 6-(2-proparglyoxyphenyl)hexanoic acid (PPOH, 5×10^{-5} mol/l; an inhibitor of CYP/epoxygenase/EET synthase) for 45 min.

Passive diameter (PD). At the conclusion of each experiment, the suffusion solution was changed to a Ca^{2+}-free PSS containing 10^{-3} M EGTA. Vessels were incubated for 10 min, and diameter was recorded at each pressure step.

LC/MS/MS-based measurements for EETs and DHETs in isolated mesenteric arteries. The mesentery was excised and perfused with PSS through a superior mesenteric artery catheter to flush away the blood. First-order mesenteric arteries were isolated and pulverized in liquid nitrogen. EETs and DHETs were extracted following alkali hydrolysis to release esterified EETs or DHETs and quantified with a Q-trap 3200 linear ion trap quadrupole LC/MS/MS equipped with a Turbo V ion source operated in negative electrospray mode (Applied Biosystems, Foster City, CA), as described previously (29, 49). NaOH (1 mol/l, 1 ml) was added to the arteries after extraction for their dissolution and determination of protein concentration (Bio-Rad, Hercules, CA). Data are presented as total EETs or DHETs that include the sum of all free and esterified four regioisomeric EETs or DHETs. Mesenteric arteries were selected for the assessment of EETs and DHETs because of their abundance and sufficient length. Also, mesenteric arteries have similar patterns of vascular response and endothelial mediators as those found in the gracilis muscle arteries (19).

Western blot analysis. Isolated single first-order mesenteric arteries were pulverized in liquid N_2. Twenty-microliter 1× Laemmli buffer was added to each sample and kept in 4°C for 30 min. Samples then underwent two bouts of sonication (1 min each), were boiled for 5 min, and centrifuged. The supernatant was then loaded on to a 10% SDS-PAGE gel and transferred to a PVDF membrane. The membrane was probed with specific primary antibodies for sEH, eNOS, (Santa Cruz Biotechnology), CYP2C29, or CYP4A (Biodesign), and appropriate secondary antibodies conjugated with horseradish peroxidase. Specific bands were visualized with a chemiluminescence kit and normalized to β-actin.

Calculation and statistical analysis. Changes in diameter in response to intraluminal pressure were reported as actual measurements in micrometers or normalized to their corresponding passive diameter (PD) and expressed as percent PD. Agonist-induced vasodilator responses were expressed as changes in diameter as percentage of PD. The myogenic index was calculated by using the formula: $100 \times \Delta r_i/r_i/\Delta P$, where r_i is the internal radius of vessels, Δr_i is the change in vessel radius in response to one step of pressure increment, and ΔP is the change in perfusion pressure (23). Data are expressed as means ± SE, and *n* refers the number of mice. Statistical analysis was performed

Table 1. *Blood pressure and heart rate of mice*

	SBP, mmHg	DBP, mmHg	MAP, mmHg	HR, beats/min
WT	114 ± 1	90 ± 3	98 ± 2	451 ± 13
sEH-KO	103 ± 2*	77 ± 2*	86 ± 2*	457 ± 21
eNOS-KO	130 ± 2*	105 ± 3*	113 ± 3*	448 ± 13
eNOS-KO (*t*-TUCB-treated)	105 ± 2†	81 ± 2†	89 ± 2†	457 ± 22

Values are means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; WT, wild type; sEH, soluble epoxide hydrolase; KO, knockout; eNOS, endothelial nitric oxide synthase. *Significant difference from WT mice; †significant difference from eNOS-KO mice ($n = 7$ for each group).

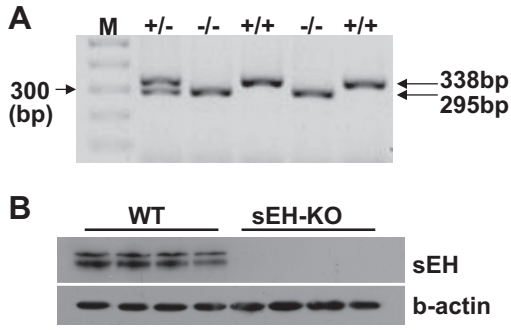

Fig. 1. *A*: examples of Ephx2 genotyping results for wild type (WT) (+/+, the band of 338 bp), heterozygous (+/−, bands of 338 and 295 bp) and homozygous (−/−, the band of 295 bp) mice. *B*: protein expression of soluble epoxide hydrolase (sEH) in mesenteric arteries of WT and sEH-knockout (KO) mice, presented in a single blot. The expression of β-actin was used for loading controls.

using repeated-measures of ANOVA, followed by the Tukey-Kramer post hoc test and Student's *t*-test. Statistical significance was accepted at a level of $P < 0.05$.

RESULTS

Reduced blood pressure in response to deficiency/inhibition of sEH. Changes in arterial blood pressure of mice as a function of the deletion of the Ephx2 gene or pharmacological inhibition of sEH are shown in Table 1. sEH-KO mice displayed significantly lower blood pressure than their WT control counterparts. Consistently, inhibition of sEH activity with *t*-TUCB prevented NO deficiency-induced hypertension in eNOS-KO mice. These data reveal a crucial role of sEH in the regulation of blood pressure.

Increased vascular EETs in sEH deficient mice. Figure 1 shows Ephx2 genotyping of offspring mice. This confirms the absence of sEH protein in blood vessels of sEH-KO mice. LC/MS/MS analysis of mesenteric arteries demonstrated increases in the levels of EETs in response to deletion of the Ephx2 gene or pharmacological inhibition of sEH. As shown in Fig. 2, vascular EET levels and the ratio of EETs/DHETs were significantly increased in sEH-KO (Fig. 2, *C* and *D*) and *t*-TUCB-treated eNOS-KO mice (Fig. 2, *E* and *F*) compared with WT and control eNOS-KO mice.

Reduced myogenic constriction in arterioles of sEH deficient mice. Figure 3*A* shows that both vascular passive diameter (PD) curves and active diameter (AD) at 20 and 40 mmHg were comparable in WT and sEH-KO mice. Upon an increase in pressure to 60 mmHg and further increases to 140 mmHg, arterioles of both strains of mice exhibited vasoconstriction. The constriction was significantly less (expressed as bigger diameter) in vessels of sEH-KO than those of WT mice, suggesting that the deficiency of sEH can attenuate pressure-induced constriction of arterioles.

Next, the role of the endothelium in the myogenic response was assessed by comparing pressure-diameter curves before (EC+) and after removal of the endothelium (EC−). Data depicted in Fig. 3 show that the removal of EC did not affect the response in vessels from WT mice but significantly shifted the pressure-diameter curve of vessels from sEH-KO mice downward (Fig. 3*A*); that is, the originally attenuated pressure-

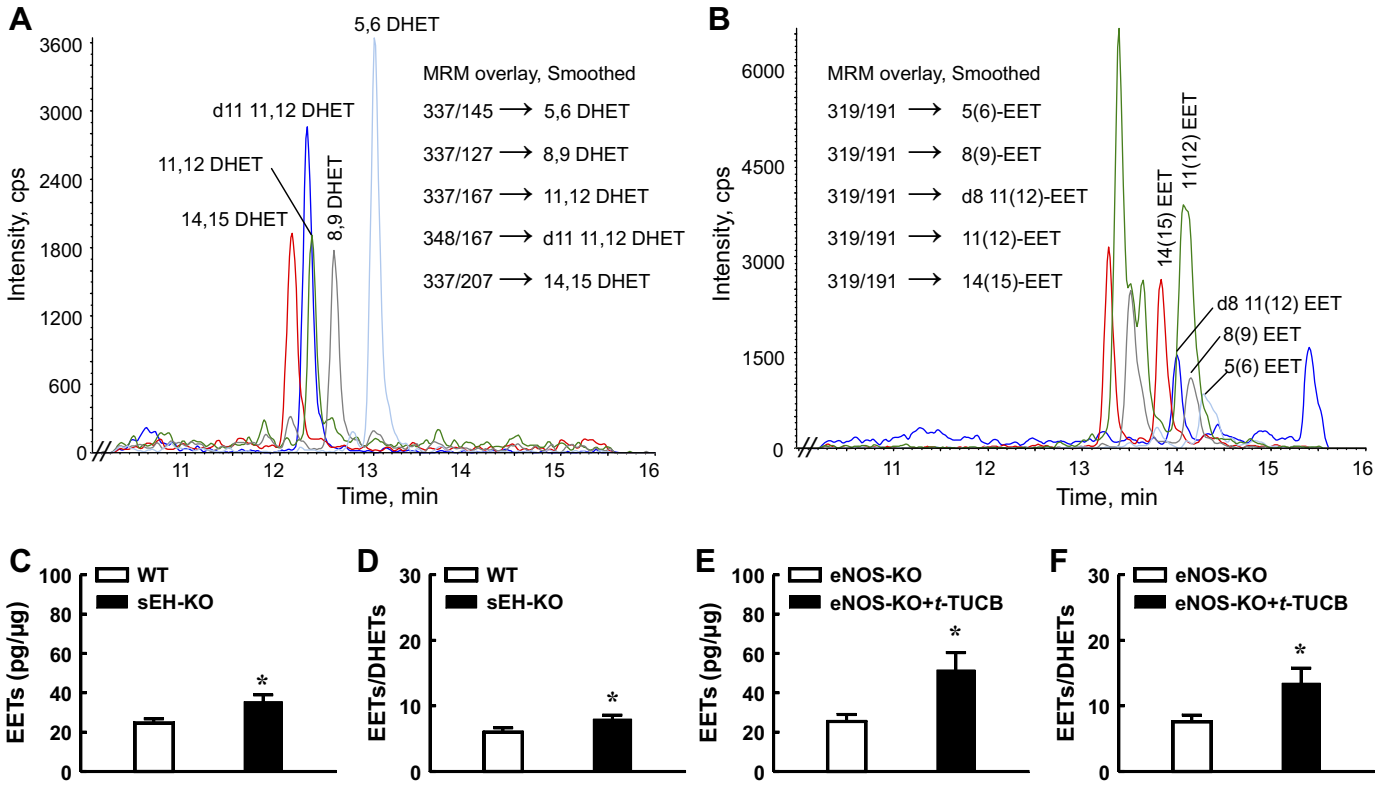

Fig. 2. Original tracing (*A* and *B*) and summary of total epoxyeicosatrienoic acids (EETs) and the ratio of EETs/dihydroxyeicosatrienoic acids (DHETs) in mesenteric arteries taken from sEH-KO and WT mice (*C* and *D*), and from endothelial nitric oxide synthase (eNOS)-KO mice treated with and without *t*-TUCB (*E* and *F*). $n = 6$ for each group. *Significant difference from WT or control eNOS-KO mice.

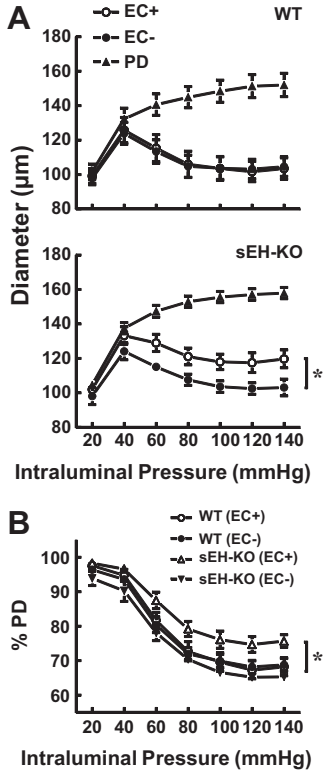

Fig. 3. *A*: diameter of gracilis muscle arterioles, as a function of intraluminal pressure, of WT ($n = 8$) and sEH-KO ($n = 8$) mice in the presence of the endothelium (EC+) and after endothelial denudation (EC−). PD stands for passive diameter. *B*: normalized myogenic responses calculated from the data shown in *A*. The diameter was normalized to the corresponding PD recorded at each pressure step. *Significant difference from intact (EC+) vessels of sEH-KO mice.

induced constriction (in the presence of EC) in vessels of sEH-KO mice returns back to the similar levels observed in vessels taken from WT controls when the endothelium was denuded. As a result, the pressure-diameter curve of EC-denuded vessels from sEH-KO mice overlapped with both curves of EC-intact and -denuded vessels from WT mice (Fig. 3*B*), indicating that the attenuation of arteriolar myogenic constriction in sEH-KO mice is dependent on an intact endothelial layer. The efficiency of endothelial denudation was confirmed by the absence of vasodilation to ACh while maintaining the dilator response to SNP (Table 2).

The myogenic index was used to assess the dynamic reaction of vessels in response to changes in pressure. Figure 4 shows comparable myogenic index (MI) curves in the endothelium-

Table 2. *Effects of endothelial removal on acetylcholine (ACh) and sodium nitroprusside (SNP)-induced dilation in gracilis muscle arterioles of mice*

	ACh (5×10^{-8} mol/l)		SNP (10^{-7} mol/l)	
	EC+	EC−	EC+	EC−
WT	20.4 ± 2.2	−0.5 ± 0.6*	12.8 ± 1.0	12.0 ± 1.1
sEH-KO	18.1 ± 1.4	−1.5 ± 1.1*	11.6 ± 0.9	12.3 ± 0.6

Data are expressed as change in diameter as a percent of passive diameter. *Significant difference in endothelium-denuded vessels (EC−) compared with endothelium-intact vessels (EC+). $n = 8$ for each group.

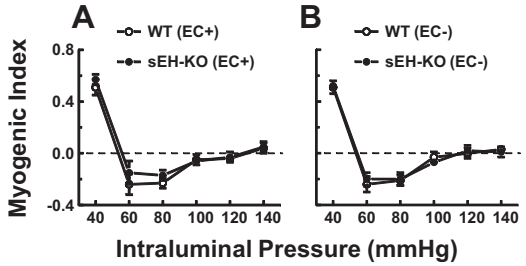

Fig. 4. Myogenic index in endothelium-intact (EC+) (*A*) and endothelium-denuded (EC−) (*B*) gracilis muscle arterioles taken from WT ($n = 8$) and sEH-KO ($n = 8$) mice.

intact (Fig. 4*A*) and -denuded (Fig. 4*B*) vessels from both WT and sEH-KO mice. This suggests that although arteriolar tone at each pressure step was different between the two strains of mice, their smooth muscle reactivity to the pressure change was identical.

In separate experiments, sEH-dependent regulation of blood pressure and myogenic constriction were evaluated in hypertensive eNOS-KO mice that had been chronically treated with *t*-TUCB (an inhibitor of sEH). As shown in Fig. 5, treatment with *t*-TUCB initiated a marked upward shift of pressure-diameter curve in vessels of eNOS-KO mice, indicating an attenuated myogenic constriction that was similar to that observed in vessels of sEH-KO mice (Fig. 3). Inhibition of EET synthesis by PPOH had no effects on pressure-diameter curves of vessels from control eNOS-KO mice, whereas it prevented the attenuated myogenic constriction caused by *t*-TUCB. Thus the important finding that emerged from these results is that the function of endothelial EETs in the modulation of myogenic response becomes predominant when their degradation is inhibited. Aligned with the attenuated myogenic constriction, blood pressure was also significantly reduced in the *t*-TUCB-treated mice compared with the control eNOS-KO mice (Table 1). As such, inhibition or deficiency of sEH is not only able to initiate hypotensive responses in normal mice, but it can also normalize blood pressure in hypertensive mice.

Effects of sEH deficiency on vascular expression of eNOS, Cyp2C29, and Cyp4A. Given that there was an endothelium-dependent attenuation of myogenic constriction in vessels from sEH-KO mice, the specific role of endothelial NO in this response was evaluated. Figure 6 shows an identical protein

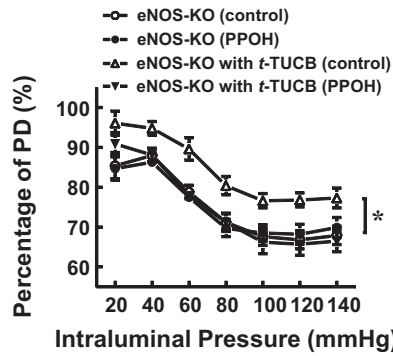

Fig. 5. Myogenic response in gracilis muscle arterioles taken from eNOS-KO mice ($n = 8$) and eNOS-KO mice treated with *t*-TUCB for 4 wk ($n = 8$), in control condition and in the presence of PPOH. *Significant difference from other curves.

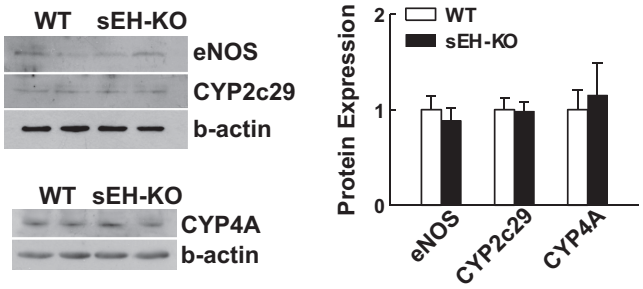

Fig. 6. Original (left panels) and summarized (right panel) data for protein expression of eNOS, CYP2C29, and CYP4A in mesenteric arteries of WT and sEH-KO mice ($n = 3$ blots).

expression of eNOS in both sEH-KO and WT mice, in line with the result showing that L-NAME did not affect vascular myogenic tone in vessels from either strain of mice (Fig. 7), implying negligible effects of NO on the responses. We did not use eNOS phosphorylation as an indicator of eNOS activity because it reflects a stimulated, but not basal release of NO (10, 12, 24). In an in vitro situation, the basal release of NO plays a major role in the control of myogenic tone. Therefore, our data exclude the possibility that changes in the basal release of NO account for the reduced myogenic constriction in vessels from sEH-KO mice. Moreover, vascular expression of CYP2C29 (an endothelial EET synthase) and CYP4A (20-HETE synthases) were also comparable in both strains of mice, suggesting that it is neither the increase in EET synthesis, nor the blunted generation of 20-HETE, but rather the reduction of EET degradation to increase in EET bioavailability that modulates the myogenic response of sEH-KO mice.

DISCUSSION

The present study provided, for the first time, direct evidence indicating that *1*) the deletion of the sEH gene or inhibition of sEH activity attenuates myogenic constriction of gracilis muscle arterioles, and *2*) the mechanism identified involves an increase in endothelial EET bioavailability that promotes vasodilation to counteract pressure-induced vasoconstriction. As a result, the balance between the two actions was tipped in favor of the dilation, leading to an attenuated arteriolar tone/resistance and, consequently, reduced blood pressure. One study has reported an EET-dependent reduction of afferent arteriolar response to the elevation of perfusion pressure of rats; it, however, was not specifically focused on EET metabolism of sEH pathway and was mainly involved in changing EET synthesis to affect the response (27).

Endothelium-dependent attenuation of myogenic response in mice deficient in sEH activity. Increases in vascular EETs and the ratio of EETs/DHETs indicated the role of sEH in the EET metabolism and the selectivity of *t*-TUCB as a sEH inhibitor (Fig. 2). In this regard, the majority of cardiovascular protective actions of pharmacological inhibition of sEH or deletion of the gene for sEH ($Ephx2^{-/-}$) have been attributed to the increased tissue and cellular EETs seen in vitro and systemic EETs in vivo (9, 25), leading to increases in vasodilation and decrease in blood pressure (33, 48). Underlying mechanisms responsible for the deletion of Ephx2 gene that resulted in decreased blood pressure are vast and have been extensively explored (6, 28); however, the specific role of the myogenic response in the sEH deficiency-induced regulation of blood pressure remains unknown. The present study indicates that in response to the stepwise increases in perfusion pressure, skeletal muscle arterioles from both WT and sEH-KO mice displayed constriction that was initiated at 60 mmHg (Fig. 3). It is important to note that the myogenic response curve in vessels of sEH-KO mice profiled a significant and paralleled upward shift compared with that of WT mice. This finding implies that an increased basal, not pressure-driven, release of vasodilator EETs contributed to the reduced myogenic constriction. This observation was supported by the identical myogenic index seen in two strains of mice (Fig. 4), suggesting the comparable reactivity of smooth muscle in response to changes of pressure. This result specifically differs from our previous findings in gracilis muscle arterioles of spontaneous hypertensive rats (SHR), in which, the myogenic constriction was proportional to the stepwise increases in pressure. As a consequence, the slope of an enhanced myogenic responsive curve of SHR significantly deviated from that of normotensive controls (20). We also found that the removal of the endothelium restored the attenuated myogenic constriction in arteries of sEH-KO, revealing an endothelium-dependent characteristic.

Increased endothelial EET bioavailability, as a function of blockade of EET degradation, counteracts the pressure-induced constriction. The pattern of blood pressure (Table 1) and vascular myogenic response in sEH-KO mice (Fig. 3) were identical to that observed in *t*-TUCB-treated eNOS-KO mice (Fig. 5). This reveals a universal characteristic for the deficient sEH activity in the regulation of vascular function and control of blood pressure. In this regard, we concluded that this regulatory mechanism is NO-independent in nature and functions in different animal models that are etiologically distinct. Interestingly, neither endothelial denudation (Fig. 3) nor inhibition of EET (Fig. 5) and NO syntheses (Fig. 7) altered the myogenic constriction in WT and control eNOS-KO mice. This suggests that generally, effects of endothelial mediators, such as NO or EETs, are minimal on controlling vascular basal tone in mice (5, 18, 21, 22, 44). This seems different from rat vasculatures, in which endothelial mediators including NO and EETs significantly affect myogenic tone (18, 21, 23, 27, 46), reflecting a species-dependent phenotype of endothelial regulatory mechanism. We also previously demonstrated that in vitro inhibition of EET synthase abolished EET-mediated shear stress- and ACh-induced vasodilation in arterioles of mice, but failed to affect basal tone of the vessels (22, 48, 49). Even

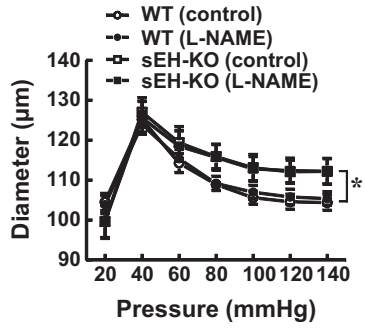

Fig. 7. Myogenic response in gracilis muscle arterioles taken from WT ($n = 5$) and sEH-KO ($n = 6$) mice before and after treatment with L-NAME (3×10^{-4} mol/l). *Significant difference between WT and sEH-KO mice.

though we reported a detectable basal release of EETs from mouse arteries in a previous study (18), the amounts of this basal release of EETs were much less than those released by stimulation with ACh or shear stress (18, 19). The EETs then, are rapidly metabolized by sEH to DHETs. Additionally, when EETs are synthesized in the endothelium, they are rapidly incorporated into membrane phospholipids, from which they are released to dilate/hyperpolarize vessels (11). Therefore, the incorporation of EETs into cell lipids is a key factor in initiating EET bioavailability. When sEH converts EETs to DHETs (4), a much smaller amount of DHETs is able to be incorporated into cell lipids as opposed to EETs (50). This may contribute to their dramatically reduced vasoactive capacity. Taken together, this could explain the failure of EETs to affect basal tone of vessels in the presence of a normal EET metabolism pathway. In this context, we interpret our data to assume that there is a favorable switch of EET metabolism from sEH hydrolysis to incorporation into membrane lipids, as a function of deficient sEH. The deficiency of sEH increases EET bioavailability without affecting EET synthesis (Fig. 6) to modulate pressure-induced constriction. The noteworthy finding is that the function of EETs seems to be discerned in most instances when their degradation is compromised. Our studies therefore highlight a physiological significance of sEH in the EET-dependent adaptation of endothelial function, in terms of inhibiting sEH as a therapeutic strategy to reduce peripheral resistance of vasculatures.

Minor contributions of 20-HETE to the control of vascular tone in response to sEH deficiency. In general, intrinsic adaptations to defects, such as genetic disruptions or pharmacological inhibition of signaling molecules, are natural responses in physiological and pathological conditions. For instance, in response to the deletion of the eNOS gene, compensatory upregulation of other endothelial mediators such as prostaglandins, EETs, and nNOS-derived NO attempt to maintain vascular homeostasis (5, 14, 18, 21, 22, 32, 46). It was noticed that there were conflicting reports pertaining to the deletion of sEH-induced changes in blood pressure, characterized as either diminishing (45) or maintaining blood pressure in sEH-KO mice (37). The latter was assumed to occur via an increase in renal 20-HETE production to compensate for the loss of Ephx2 gene. In the present study, however, sEH-KO mice displayed a hypotensive phenotype (Table 1) that could be, at least in part, attributed to the reduction in pressure-induced myogenic constriction of arterioles. We also reported a similar expression of CYP4A in vessels from sEH-KO and WT mice, and undetectable changes in their plasma and vascular 20-HETE levels (data not shown). These results provide supporting evidence for the normalization, but not enhancement of arteriolar myogenic constriction in sEH-KO and *t*-TUCB-treated mice when their endothelium was denuded (Fig. 3), or EET synthase was inhibited (Fig. 5). Thus it is unlikely that the alternation of 20-HETE signaling is involved in the responses.

Perspectives. Ephx2 is a susceptibility gene for hypertension-associated heart failure in rodents (39), and likewise, an association between single-nucleotide polymorphisms (SNPs) in Ephx2 gene and cardiovascular diseases (56). For instance, the K55R allele is associated with a higher risk of hypertension and ischemic stroke, as well as coronary heart disease (15). Interestingly, while the R287Q allele had been reported to be related with coronary artery calcification in young adults and insulin resistance in type 2 diabetic patients (13, 40), the sequential studies clarified that R287Q was not associated with hypertension (7) nor with ischemic injury, specifically myocardial infarction and stroke (36). Moreover, the R287Q mutation in African Americans exhibits a lower vascular resistance due to an increased forearm vasodilator response to bradykinin (35). Furthermore, neuronal cells transfected with the R287Q mutant display an improved survival rate after ischemic injury (31). Notably, one of the mechanisms responsible for the protective property of the R287Q mutation can be attributed to the reduced sEH activity (31), which therefore provides genotypic evidence for the correlation between sEH action and vascular function. Indeed, administration of sEH inhibitors or deletion of the Ephx2 gene significantly lowers blood pressure in various hypertensive models, including salt-sensitive hypertension (25), SHR (55), and angiotensin II-induced hypertension (30), via mechanisms involving potentiation of vasodilation, anti-inflammatory, and anti-oxidative properties. In the present study we identified a novel mechanism of myogenic regulation of arteriolar tone and control of blood pressure in response to deficient sEH. This study provides a mechanistically based rationale for the development of therapeutic interventions that trigger sEH to function as a myogenic regulator to attenuate arteriolar tone, reduce peripheral resistance, and lower blood pressure.

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DISCLOSURES

S. H. Hwang and B. D. Hammock are authors of University of California patients on soluble epoxide hydrolase inhibitor chemistry and blood pressure regulation.

AUTHOR CONTRIBUTIONS

Author contributions: D.S. and A.H. conception and design of research; D.S., K.G., and A.H. performed experiments; D.S. and A.H. analyzed data; D.S., A.J.C., S.H.H., B.D.H., M.L.S., and A.H. interpreted results of experiments; D.S. and A.H. prepared figures; D.S. and A.H. drafted manuscript; D.S., A.J.C., S.H.H., B.D.H., M.L.S., and A.H. edited and revised manuscript; D.S., A.J.C., K.G., S.H.H., B.D.H., M.L.S., and A.H. approved final version of manuscript.

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