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Increases in plasma *trans*-EETs and blood pressure reduction in spontaneously hypertensive rats

Houli Jiang,¹ John Quilley,¹ Anabel B. Doumad,¹ Angela G. Zhu,¹ John R. Falck,² Bruce D. Hammock,³ Charles T. Stier, Jr.,¹ and Mairead A. Carroll¹

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Jiang H, Quilley J, Doumad AB, Zhu AG, Falck JR, Hammock BD, Stier CT, Jr, Carroll MA. Increases in plasma *trans*-EETs and blood pressure reduction in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 300: H1990–H1996, 2011. First published March 11, 2011; doi:10.1152/ajpheart.01267.2010.—Epoxyeicosatrienoic acids (EETs) are vasodilator, natriuretic, and antiinflammatory lipid mediators. Both *cis*- and *trans*-EETs are stored in phospholipids and in red blood cells (RBCs) in the circulation; the maximal velocity (V_{\max}) of *trans*-EET hydrolysis by soluble epoxide hydrolase (sEH) is threefold that of *cis*-EETs. Because RBCs of the spontaneously hypertensive rat (SHR) exhibit increased sEH activity, a deficiency of *trans*-EETs in the SHR was hypothesized to increase blood pressure (BP). This prediction was fulfilled, since sEH inhibition with *cis*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (AUCB; 2 mg·kg⁻¹·day⁻¹ for 7 days) in the SHR reduced mean BP from 176 ± 8 to 153 ± 5 mmHg ($P < 0.05$), whereas BP in the control Wistar-Kyoto rat (WKY) was unaffected. Plasma levels of EETs in the SHR were lower than in the age-matched control WKY (16.4 ± 1.6 vs. 26.1 ± 1.8 ng/ml; $P < 0.05$). The decrease in BP in the SHR treated with AUCB was associated with an increase in plasma EETs, which was mostly accounted for by increasing *trans*-EET from 4.1 ± 0.2 to 7.9 ± 1.5 ng/ml ($P < 0.05$). Consistent with the effect of increased plasma *trans*-EETs and reduced BP in the SHR, the 14,15-*trans*-EET was more potent (ED₅₀ 10⁻¹⁰ M; maximum dilation 59 ± 15 μm) than the *cis*-isomer (ED₅₀ 10⁻⁹ M; maximum dilation 30 ± 11 μm) in relaxing rat precontracted arcuate arteries. The 11,12-EET *cis*- and *trans*-isomers were equipotent dilators as were the 8,9-EET isomers. In summary, inhibition of sEH resulted in a twofold increase in plasma *trans*-EETs and reduced mean BP in the SHR. The greater vasodilator potency of *trans*- vs. *cis*-EETs may contribute to the antihypertensive effects of sEH inhibitors.

epoxyeicosatrienoic acids; soluble epoxide hydrolase; hypertension; cytochrome *P*-450

EPOXYEICOSATRIENOIC ACIDS (EETs) are vasodilator, natriuretic, and anti-inflammatory lipid mediators (5, 6, 16, 30, 53). In the circulation, EETs are primarily esterified in plasma phospholipids and stored in red blood cell (RBC) membranes (22, 27, 41). While lipid peroxidation can produce *cis*- and *trans*-EETs (26, 27), this is unlikely a direct source because of the presence of multiple antioxidant systems in vivo (19, 47). NADPH-dependent cytochrome *P*-450 (CYP) metabolism of arachidonic acid produces only *cis*-EETs (8, 50, 56), whereas hydroperoxide-dependent CYP cooxidation of arachidonic acid can result in formation of both *cis*- and *trans*-EETs (4, 7, 52).

Indeed, we have found CYP isoforms stimulated by lipid hydroperoxides preferentially produce *trans*- over *cis*-EETs (Jiang, unpublished observations), and we were the first to report the presence of *trans*-EETs in vivo (26, 27). Isomers of *cis*- and *trans*-EETs possess an equipotent platelet antiaggregatory activity (17, 26), but both 5,6-*trans*-EET and its hydration product 5,6-*erythro*-dihydroxyeicosatrienoic acids (DHET) exhibited greater vasodilator effects than 5,6-*cis*-EET (26).

EETs are antihypertensive, since increased endothelial epoxide hydrolase expression significantly reduced blood pressure (BP) in hypertensive mice (32), and intra-arterial or intravenous infusion of 14,15-EET reduced BP in the spontaneously hypertensive rat (SHR) by a maximum of 45 mmHg (36). Moreover, inhibition of EET production by clotrimazole increased BP by 23 mmHg in Dahl rats fed a high-salt diet (38). EETs are hydrolyzed by soluble epoxide hydrolase (sEH) to DHETs, which generally have less biological activity than EETs. Increased sEH activity has been associated with hypertension (1, 18, 24, 55). Inhibition of EET hydrolysis by sEH inhibitors reduced BP by 20 to 30 mmHg in rats with angiotensin II (ANG II)-salt-induced hypertension (21, 24, 37), as well as in the SHR (55) and the stroke-prone SHR (34). Deletion of the sEH gene reduced BP by 16 mmHg compared with wild-type DOCA-salt hypertensive mice (39). Inhibition of sEH is a potential therapeutic approach for control of BP, vascular inflammation, and for providing cardiac and renal protection by raising levels of EETs in vivo (45, 49).

RBCs represent a major source of sEH in the circulation for the hydration of *cis*- and *trans*-EETs, producing *threo*- and *erythro*-DHETs, respectively (29). The maximal velocity (V_{\max}) for the hydrolysis of *trans*-EETs is approximately three times greater than for their respective *cis*-isomers (29). The 14,15-*trans*-EET is hydrolyzed the fastest by rat RBCs among all EET isomers, and the rate of EET hydrolysis decreased two- to threefold sequentially from 14,15-, 11,12-, and 8,9- to 5,6-EETs (29). Inhibition of sEH will favor the preservation of *trans*-EETs over *cis*-EETs, since *trans*-EETs are hydrolyzed three times more rapidly than *cis*-EETs by sEH, and the Michaelis constant for *trans*- vs. *cis*-EETs is on average 1.6 vs. 1.0 μM, respectively (29). There is greater sEH expression and activity in SHR than in the normotensive Wistar-Kyoto rat (WKY) control strain (18, 48, 55).

RBCs are also reservoirs and carriers of EETs in the circulation (22, 27). RBCs, in addition to O₂ delivery in the microvasculature, participate in the regulation of microvascular tone by releasing vasoactive factors, including EETs (25, 28). The role of RBC sEH in the regulation of circulating EETs may be particularly significant when considering that there are

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effects of EETs on the rheological and hemodynamic determinants of the circulation. Inhibition of RBC sEH presumably contributes to elevating effects of EETs on regional blood flows to a greater degree than inhibition of sEH localized "in the smooth muscle layers of the arterial wall" (54). The present studies focused on effects of the potent sEH inhibitor, *cis*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (AUCB) (23, 29), on BP and EET levels in the circulation in relation to the activity of RBC sEH in the SHR compared with the WKY, as well as the direct vasorelaxant activity of *cis*- vs. *trans*-EETs in renal microvessels.

MATERIALS AND METHODS

Animals. The experimental procedures in animal use were approved by the Institutional Animal Care and Use Committee of New York Medical College and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the United States National Institutes of Health. Ten-week-old male SHR and WKY rats were purchased from Taconic Farms. Seven-week-old male Sprague-Dawley (SD) rats were from Charles River Laboratories. Rats were maintained at 22°C with alternating cycles of light and darkness and fed ad libitum with standard rat chow (Purina) and water. AUCB (0.01 mg/ml to provide a dose of 2 mg·kg⁻¹·day⁻¹) was dissolved in drinking water containing 0.2% ethanol and administered to 11-wk-old SHR and WKY rats for 7 days while the vehicle control rats were given tap water containing 0.2% ethanol.

BP measurements. Noninvasive BP measurements were made using a volume-pressure tail-cuff system (Kent Scientific) (15). Each rat was subjected to five acclimation cycles followed by five measurement cycles for BP readings. In some cases, BP was measured by radiotelemetry (35) to confirm the changes measured by tail cuff.

Reagents. Standard racemic *trans*-EETs were synthesized as described, and the purity as analyzed by HPLC was over 99% (14, 26). Standard *cis*-EETs were from Cayman Chemical (Ann Arbor, MI). AUCB was provided by Dr. Hammock and has been characterized as a potent sEH inhibitor (23, 29). BSA and HPLC-grade organic solvents were obtained from Fisher Scientific (Pittsburgh, PA), and EET-d8 standards were from Enzo Life Sciences (Plymouth Meeting, PA). Other reagents were purchased from Sigma (St. Louis, MO).

RBC preparation and incubation. Preparation and incubation of rat RBCs was performed as previously described (29). Briefly, up to 10 ml blood were drawn from the inferior vena cava of pentobarbital-anesthetized rats. RBCs were isolated by centrifugation at 400–800 *g* and then washed with cold physiological salt solution after the buffy layer was discarded. RBC incubations to compare the hydrolysis of EETs by rat RBCs were carried out using 16 ng of *cis*- or *trans*-EETs in 50 μ l prewarmed 5 \times 10⁸ RBCs in PBS at 37°C for 10 min with shaking at 600 rpm in a VWR 3-mm orbital shaker.

Plasma and urine EET extraction. Rat plasma was obtained after centrifuging blood at 2,000 *g* for 10 min and mixed with polymer-bound triphenylphosphine (TPP, 1 mg/ml) to quench free radical-induced lipid peroxidation. Phospholipid was extracted from 0.4 ml plasma using the Blich-Dyer (3) method and hydrolyzed with 1 M NaOH for 90 min at room temperature. The hydrolysis mixture was then neutralized with 1 M HCl and extracted two times with 2 ml ethyl acetate. The ethyl acetate extract was dried under a gentle stream of nitrogen and dissolved in acetonitrile (20 μ l) for immediate LC/MS/MS analysis (27).

Rat urines (24 h) were collected in tubes containing 5 mg polymer-bound TPP. Urine samples (2 ml) with added d11-labeled 8,9- and 14,15-DHET and d8-labeled 8,9-, 11,12-, and 14,15-EET (1 ng each) were vigorously mixed two times with 3 ml hexane-ethyl acetate (1:1) to extract EETs and DHETs. The combined organic phase was backwashed with 4 ml of water, dried under a gentle stream of N₂, and dissolved in 80 μ l acetonitrile for HPLC separation and GC/MS analysis as described (26, 41).

Mass spectrometry analyses. ESI LC/MS/MS analyses of EETs and DHETs were carried out as described (27, 29). Briefly, a Finnigan LCQ Advantage quadrupole ion-trap mass spectrometer equipped with ESI source run by XCALIBUR software was used. MS/MS breakdown for mass-to-charge ratio (*m/z*) 319 was at an energy level of 35% set by the instrument, and a seven-point Gaussian smoothing was applied in the mass data processing. Quantification of individual EETs was based on standard curves (*r* = 0.99) between their respective characteristic fragmentation ions with reference to an internal standard of 2 ng of d8-11,12-EET.

For quantification using electron-capture negative-chemical ionization GC/MS, purified DHET samples were derivatized to trimethylsilyl ether pentafluorobenzyl (PFB) esters, and EETs were derivatized to PFB esters as described (26, 28). The ions *m/z* of 481 and 492 were monitored for endogenous and d11-labeled DHETs; the ions *m/z* of 319 and 327 were monitored for endogenous and d8-labeled EETs.

Western blot analysis of sEH. RBC cytosol was obtained by centrifugation of lysed RBCs at 10,000 *g* for 1 h and then diluted 1:2 with 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 1% SDS on ice. Total protein concentration was quantified with the Pierce BSA assay using Fraction V BSA as the calibrating standard. For each sample, 50 μ g of protein were loaded on a 12% SDS-PAGE, and Western blot analysis was carried out as described (29). Expression of GAPDH was detected using a monoclonal mouse antibody and a goat anti-mouse IgG labeled with horseradish peroxidase. Bands were visualized using the ECL kit from Amersham and results calculated as a ratio relative to GAPDH expression.

Rat renal arcuate artery studies. Activities of *cis*- and *trans*-EET regioisomers were evaluated in microdissected SD rat arcuate arteries (~100 μ m internal diameter) that were cannulated and pressurized to 80 mmHg as described (12). Arcuate arteries were chosen because of their exquisite sensitivity to *cis*-EETs (12). We determined that neither indomethacin (10 μ M), a cyclooxygenase inhibitor, nor L-NAME (200 μ M), a nitric oxide synthase inhibitor, affected the vasoactivity of *cis*- and *trans*-EETs (data not shown). The vessels were precontracted with phenylephrine (10⁻⁷ mol/l), and cumulative concentration responses to individual EET regioisomers were recorded before and after application of 14,15-EEZE (10⁻⁶ mol/l), a selective EET antagonist (20). We also examined the activity of the 14,15-EET isomers on arcuate arteries obtained from 12-wk-old SHR and WKY rats. In these experiments, arteries were pressurized to 80 and 120 mmHg to address any effects of different baseline pressures that exist in SHR and WKY rats.

Data analysis. Results are presented as means \pm SE and analyzed using GraphPad Prism 5 (San Diego, CA) software. Comparisons among multiple groups were made by ANOVA followed by Tukey's test for differences between groups. Paired and unpaired *t*-tests were used for comparison between two groups where appropriate. A *P* value <0.05 was considered as statistically significant.

RESULTS

Increased RBC sEH activity and expression in SHR compared with WKY. We examined RBC sEH activity and expression in SHR and WKY rats, since elevated sEH expression has been reported to occur in both the kidney and the brain of SHR (18, 48, 55). Incubation of *cis*- and *trans*-isomers of 14,15-, 11,12-, and 8,9-EETs (1 μ M each) with RBCs from the 11-wk-old SHR and WKY revealed significant differences in the hydrolysis of EETs. Specific rates of EET hydrolysis by RBCs of the SHR are on average more than double that of the WKY (Fig. 1A). Greater hydrolysis of *trans*- than *cis*-EETs by RBCs from both the WKY and the SHR was also found (Fig. 1A), which is consistent with a *V*_{max} for the hydrolysis of *trans*-EETs that is three times greater than that of *cis*-EETs (29).

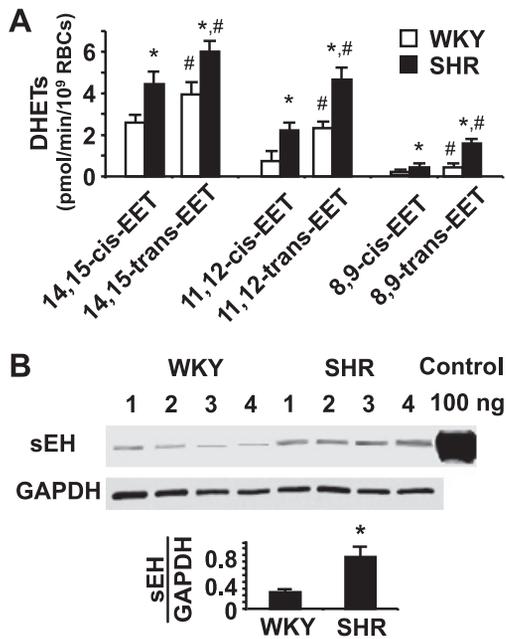


Fig. 1. Increased soluble epoxide hydrolase (sEH) activity and expression in red blood cells (RBCs) of the spontaneously hypertensive (SHR) vs. the Wistar-Kyoto (WKY) rat. A: hydrolysis of epoxyeicosatrienoic acids (EETs) by RBCs from the SHR and the WKY at 37°C for 10 min. DHET, dihydroxyeicosatrienoic acid. **P* < 0.05 compared with RBCs from the WKY for hydrolysis of the same EET isomer, *n* = 6. #*P* < 0.05 compared with hydrolysis of the *cis*-isomer, respectively, *n* = 6. B: Western blot analysis of sEH in the RBC cytosol from the WKY and the SHR. **P* < 0.05 compared with the WKY, *n* = 4.

The greater RBC sEH activity in SHR compared with WKY is consistent with the increased expression of sEH in the RBC cytosol of the SHR compared with the WKY (Fig. 1B). These results support previous reports indicating increased sEH expression and activity in hypertension (1, 24, 48, 55). Increased sEH activity has been postulated to be responsible for reduced plasma EET levels in essential hypertension (41).

Changes in plasma and urinary EETs and DHETs with sEH inhibition. In 12-wk-old SHR treated for a week with AUCB, total plasma EETs in the SHR increased 22%, mostly reflecting an increase in plasma *trans*-EETs (Table 1). Plasma total EETs in the WKY remained unchanged with AUCB treatment (26.1 ± 1.8 vs. 28.5 ± 3.2 ng/ml). Total plasma EETs were higher in untreated WKY than SHR rats (26.1 vs. 16.4 ng/ml), but a significant elevation in response to sEH inhibition was only seen in the SHR (Table 1).

Total plasma *cis*-EETs were twofold greater than *trans*-EETs in control WKY rats (18.3 vs. 7.8 ng/ml). AUCB treatment did not affect plasma total *cis*-EET concentrations in the WKY and the SHR (Table 1). However, AUCB increased plasma *trans*-EET levels similarly in both the SHR (4.1–7.9 ng/ml) and WKY (7.8–12.5 ng/ml), viz., ~4 ng/ml, which had the greater impact on plasma *trans*-EETs in the AUCB-treated SHR, given the relatively low total plasma EETs of the SHR (Table 1). Except for 5,6-*trans*-EET, a poor substrate for the sEH (10, 29), other *trans*-EETs were increased significantly in both strains when treated with the sEH inhibitor (Table 1).

Rat plasma total DHETs were ~10% of the level of total EETs. A decrease in plasma DHETs was not found as expected with sEH inhibition (vehicle vs. AUCB-treated rats: 2.4 ± 0.6

vs. 2.7 ± 0.6 ng/ml for WKY; 1.7 ± 0.2 vs. 2.0 ± 0.3 ng/ml for SHR), possibly reflecting a dynamic balance among formation, metabolism, and excretion (51).

Total EETs and DHETs in rat urine were analyzed by GC/MS. Rat urinary excretion was mostly in the form of DHETs, whereas EETs accounted for <20% of the total. AUCB treatment for 7 days did not affect the urinary excretion of EETs or DHETs in either SHR or WKY rats. However, the total urinary excretion of EETs and DHETs was ~50% less in SHR than in WKY on all days measured (for example, on day 7, DHET excretion with vehicle vs. AUCB was 42 ± 4 vs. 40 ± 5 for WKY and 27 ± 4 vs. 24 ± 3 ng/day for SHR, *n* = 8).

AUCB administration inhibits RBC hydrolysis of EETs. To examine the effect of AUCB treatment on sEH activity, RBCs from the control and treated WKY and SHR were separated and tested for the hydrolysis of 1 μM 14,15-*cis*- and 14,15-*trans*-EET. RBCs from AUCB-treated rats exhibited a greatly reduced capacity to hydrolyze *cis*- and *trans*-EETs; hydrolysis of 14,15-*trans*-EET by RBCs of the AUCB-treated SHR was inhibited by 60.0 ± 3.2% (*n* = 8), whereas that of 14,15-*cis*-EET was inhibited by 41.1 ± 1.9% (*n* = 6) compared with RBCs from the vehicle SHR; similar percent inhibition was also found in the WKY (Table 2), consistent with the greater increase in *trans*-EETs compared with *cis*-EETs on sEH inhibition.

RBCs from the vehicle-treated SHR showed the greatest capacity to generate both *erythro*- and *threo*-DHETs (7.02 ± 0.70 and 4.40 ± 0.53 pmol·min⁻¹·10⁻⁹ RBCs, respectively), in agreement with elevated expression of sEH in the SHR (Fig. 1B). Treatment of the SHR with AUCB reduced its RBC hydrolysis of *trans*-EETs, resulting in equalization of *erythro*- and *threo*-DHET production, 2.81 vs. 2.59 pmol·min⁻¹·10⁻⁹ RBCs, respectively (Table 2). The lesser production of DHETs

Table 1. Plasma concentrations of individual *cis*- and *trans*-EETs in WKY and SHR treated with vehicle or AUCB for 1 wk

	WKY		SHR	
	Vehicle	AUCB	Vehicle	AUCB
14,15- <i>cis</i> -EET	5.8 ± 0.6	5.2 ± 0.7	4.5 ± 0.6	4.4 ± 0.7
14,15- <i>trans</i> -EET	1.6 ± 0.2	3.4 ± 0.6*	1.1 ± 0.1	2.3 ± 0.7*
11,12- <i>cis</i> -EET	2.1 ± 0.3	2.7 ± 0.3	1.4 ± 0.3	1.6 ± 0.2
11,12- <i>trans</i> -EET	2.0 ± 0.2	3.6 ± 0.8*	0.9 ± 0.1	2.0 ± 0.5*
8,9- <i>cis</i> -EET	4.9 ± 0.4	3.3 ± 0.4	3.2 ± 0.4	3.0 ± 0.3
8,9- <i>trans</i> -EET	1.8 ± 0.3	2.7 ± 0.7*	0.9 ± 0.1	1.9 ± 0.3*
5,6- <i>cis</i> -EET	5.5 ± 0.4	4.8 ± 0.3	3.2 ± 0.4	3.1 ± 0.2
5,6- <i>trans</i> -EET	2.4 ± 0.1	2.8 ± 0.5	1.2 ± 0.1	1.7 ± 0.3
Total <i>cis</i> -EET	18.3 ± 1.4	16.0 ± 1.5	12.3 ± 1.6	12.1 ± 1.3
Total <i>trans</i> -EET	7.8 ± 0.5	12.5 ± 1.9*	4.1 ± 0.2	7.9 ± 1.5*
Overall EETs	26.1 ± 1.8	28.5 ± 3.2	16.4 ± 1.6	20.0 ± 2.4*

Values were determined by LC/MS/MS and expressed as means ± SE (ng/ml); *n* = 6–8 rats in each group. EET, epoxyeicosatrienoic acid; AUCB, *cis*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid. Eleven-week-old Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were treated with vehicle or AUCB (2 mg · kg⁻¹ · day⁻¹ in the drinking water) for 7 days. **P* < 0.05 compared with plasma concentrations of vehicle rats of the same strain.

Table 2. Conversion of 14,15-*cis*-EET to 14,15-*threo*-DHET and 14,15-*trans*-EET to 14,15-*erythro*-DHET by RBCs of vehicle- and AUCB-treated WKY and SHR

Rat RBCs	DHET Produced, pmol · min ⁻¹ · 10 ⁻⁹ RBCs	
	14,15- <i>threo</i> -	14,15- <i>erythro</i> -
WKY vehicle	2.82 ± 0.34	4.37 ± 0.52#
WKY AUCB	1.67 ± 0.20*	1.84 ± 0.22*
SHR vehicle	4.40 ± 0.53	7.02 ± 0.70#
SHR AUCB	2.59 ± 0.31*	2.81 ± 0.34*

Values are means ± SE; *n* = 6-8 rats in each group. DHET, dihydroxyicosatrienoic acid; RBC, red blood cell. 14,15-*cis*- and 14,15-*trans*-EET (1 μM each) were added to RBCs (5 × 10⁸ RBCs in 50 μl PBS) of the vehicle control and the AUCB-treated SHR and WKY for 10 min at 37°C. **P* < 0.05 compared with vehicle control rat RBCs for hydrolysis of the same 14,15-EET isomer. #*P* < 0.05 compared with the hydrolysis of 14,15-*cis*-EET by the same RBCs.

by vehicle-treated WKY RBCs compared with that of the vehicle-treated SHR once again reflects differences in RBC sEH activity in the two strains. As was the case for SHR, sEH inhibition in WKY resulted in equalization of *erythro*- and *threo*-DHET production, viz., 1.84 vs. 1.67 pmol·min⁻¹·10⁻⁹ RBCs, respectively (Table 2).

BP reduction in the SHR is associated with increases in plasma *trans*-EETs. Mean arterial BP fell from 176 ± 8 to 153 ± 5 mmHg in the SHR, whereas it was unaffected in the WKY after 1 wk of treatment with AUCB (Fig. 2A). The reduction in BP with AUCB treatment in SHR reached a maximum in a week although BP was significantly reduced by day 3. Continued AUCB treatment did not result in a further decrease in BP. We anticipate that the effect of AUCB was maximal based on other studies of the potency of AUCB in inhibiting sEH (23, 29). The heart rate was not changed with AUCB treatment in either WKY rats or SHR (Fig. 2B).

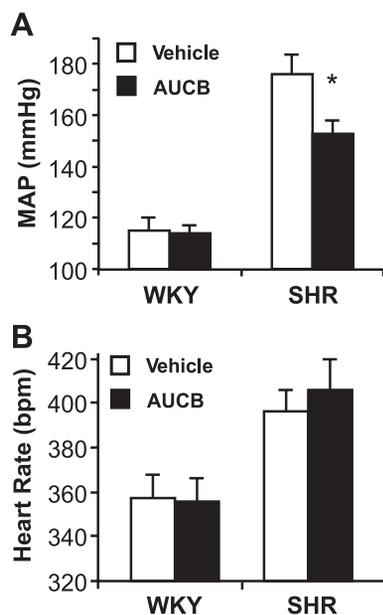


Fig. 2. Effects of 1 wk oral administration of *cis*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (AUCB) on mean arterial blood pressure (MAP, A) and heart rate (B). **P* < 0.05 compared with vehicle-treated WKY or SHR of the same strain, *n* = 6-8.

The BP reduction is consistent with significant increases in plasma *trans*-EET concentrations in the SHR (Table 1). Although inhibition of sEH also increased *trans*-EETs in the plasma of normotensive WKY rats, the relative increase was less than that for the SHR and was not associated with a reduction in BP, which is not surprising when considering that most antihypertensive agents exert little or no effect on BP in normotensive individuals. The results suggest that the biological activities of *trans*-EETs may have contributed to the fall in the elevated BP in the SHR. There is also evidence of enhanced sensitivity of the renal vasculature to the vasodilator action of EETs in the SHR (43).

Comparison of *cis*- vs. *trans*-EETs on relaxation of precontracted rat arcuate arteries. Because only *trans*-EETs were increased by treatment with AUCB, we compared the activity of *cis*- and *trans*-EETs on a precontracted rat arcuate artery preparation (Fig. 3). We found that the 14,15-*trans*-EET was more potent (ED₅₀ 5 × 10⁻¹¹ M; 59 ± 15 μm dilation at 10⁻⁷M) than 14,15-*cis*-EET. The 11,12-EET *cis*- and *trans*-isomers were equipotent dilators (ED₅₀ 10⁻¹⁰ M) as were the 8,9-EET isomers (ED₅₀ 5 × 10⁻¹⁰ M). The vasodilator activity of EETs, except for 8,9-EETs, was significantly reduced by the presence of 14,15-EEZE (10⁻⁶ mol/l), an EET antagonist (20). The responses to 8,9-*cis*-EET were unaffected by preincubation with 14,15-EEZE, whereas the potency of 8,9-*trans*-EET was significantly enhanced in the presence of 14,15-EEZE.

Because the 14,15-EET isomers exhibited the major difference in dilator activity, we compared the activity of 14,15-*trans*-EET and 14,15-*cis*-EET in arteries obtained from SHR and WKY rats pressurized to 80 and 120 mmHg. We observed

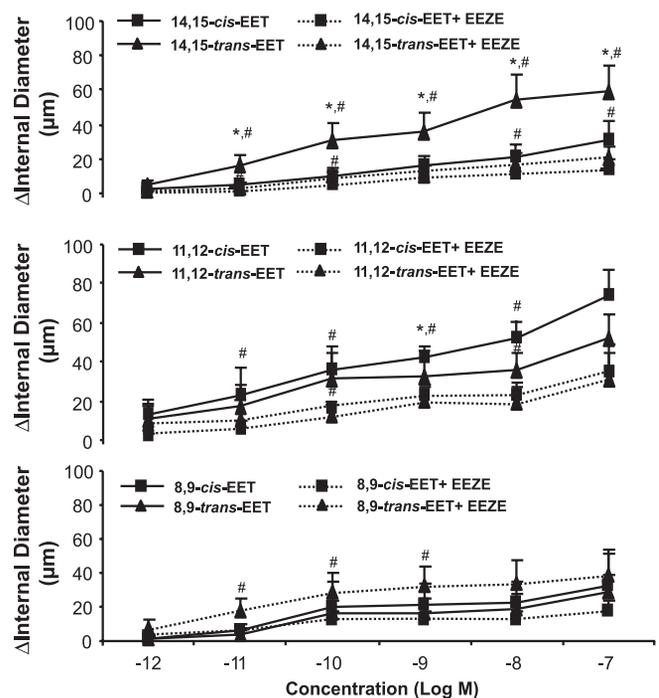


Fig. 3. Comparison of the vasoactivity of *cis*- and *trans*-EETs on pressurized rat arcuate arteries. Arteries were pressurized to 80 mmHg and precontracted with phenylephrine. Concentration-response curves to either *cis*- or *trans*-EETs were repeated in the presence of 14,15-EEZE (EEZE). **P* < 0.05 compared with responses to respective *cis*-EET, *n* = 6-8. #*P* < 0.05 compared with the vasodilator activity of EETs and effects of EEZE, *n* = 6-8.

no difference in basal diameters, sensitivity to phenylephrine, or responsiveness to EETs between SHR and WKY arteries. The 14,15-*trans*-EET was more potent than 14,15-*cis*-EET in both SHR and WKY arteries. No differences in the activities of 14,15-*trans*-EET and 14,15-*cis*-EET were apparent at 80 mmHg (Fig. 4) or 120 mmHg (data not shown).

DISCUSSION

The major new findings from this study are: 1) that plasma levels of EETs, especially *trans*-EETs, are reduced in the SHR; 2) the reduction in BP in SHR in response to sEH inhibition is associated with increased levels of plasma *trans*-EETs; and 3) the renal microvascular vasodilator activity of 14,15-*trans*-EET is greater than 14,15-*cis*-EET. In addition, the finding of increased expression and activity of sEH in RBCs of SHR compared with WKY is consistent with earlier studies showing increased sEH activity in hypertension (1, 18, 24, 55). This study also confirms that *trans*-EETs are hydrolyzed faster than *cis*-EETs by sEH (29), that inhibition of sEH lowers BP in SHR (34, 55), and that plasma EET levels are reduced in hypertension (41). When considered together, our results support the concept that an increase in plasma *trans*-EETs contributes to the antihypertensive effect of inhibition of sEH in SHR. Unequivocal evidence for a role of *trans*-EETs in the hypotensive effect of AUCB to inhibit sEH will require the demonstration that specific inhibition of the generation or activity of *trans*-EETs can prevent the hypotensive effect of AUCB in the SHR, a challenging task requiring elucidation of the mechanism of *trans*-EET formation and development of specific antagonists against the action of *trans*-EETs.

This study points to a potential role of RBCs, and specifically the sEH activity of RBCs, in the regulation of BP. Whether this is linked to the antihypertensive effect of phlebotomy (2, 13) or embryo transfer (33) deserves further investigation. Similarly, it would be of great interest to assess any effects on BP resulting from transfusion of RBCs from SHR to WKY rats. This present study identified increases mostly in plasma *trans*-EETs with AUCB treatment, but more profound increases in *cis*- and *trans*-EETs in specific vascular beds cannot be excluded.

SHR features increased oxidative stress (11, 46), and the lipid hydroperoxide-dependent CYP metabolism should result in elevated *trans*-EET formation. However, plasma *trans*-EET

levels are significantly lower in SHR compared with WKY and may reflect a deficiency in the formation of *trans*-EETs by SHR. The preferred hydrolysis of *trans*-EETs by sEH may also account for the deficit of *trans*-EETs in the plasma of SHR vs. WKY, since sEH expression and activity are increased more than twofold in RBCs from the SHR. Consequently, we envisaged that inhibition of sEH would increase levels of *trans*-EETs more than those of *cis*-EETs and that the increase in *trans*-EETs would be relatively greater for the SHR. Both of these predictions proved correct; *trans*-EETs were increased in the plasma of both the WKY and the SHR following treatment with AUCB, whereas there was no change in total plasma *cis*-EETs; levels of *trans*-EETs in the plasma of the SHR were increased twofold compared with only a 50% increase in WKY. These results indicate a potential role for *trans*-EETs in the hypotensive effect of AUCB in the SHR, an idea that is further supported by the greater or equal renal vasodilator activity of *trans*- vs. *cis*-EETs (Fig. 3).

EETs have direct actions to relax vascular smooth muscle, presumably via activation of potassium channels (5, 31), and EETs may also potentiate the dilator effects of other hormones (5, 30). Consequently, an increase in *trans*-EETs in response to inhibition of sEH might account for the hypotensive effect of AUCB in SHR through direct and indirect mechanisms. Of the EET isomers, 14,15-*trans*-EET and 11,12-*cis*-EET were equipotent vasodilators. The 14,15-EET isomers exhibited the greatest differences in potency with the 14,15-*trans*-EET being a more potent renal vasodilator than 14,15-*cis*-EET. Falck et al. (14) have reported less potency for 14,15-*trans*- vs. 14,15-*cis*-EET in bovine coronary arteries (14), and this discrepancy should be of no surprise considering the different potencies of EETs in various vascular beds and species (5, 9, 12, 44). However, in isolated arcuate arteries obtained from SHR and WKY rats, the greater vasodilator responses to 14,15-*trans*- compared with 14,15-*cis*-EET were consistent with those obtained from normotensive SD rats, and no difference in responsiveness was observed between arteries from SHR and WKY rats. This is in contrast to the results obtained in the isolated perfused kidney of SHRs where vasodilator responses to 5,6-EET and its precursor, arachidonic acid, were markedly enhanced compared with those of control rats (42, 43). The vasodilator activity of the *cis*- and *trans*-EETs, except for 8,9-EETs, was attenuated in the presence of the putative EET antagonist 14,15-EEZE, suggesting a common mechanism for vasorelaxation. In contrast, the responses to 8,9-*cis*-EET were not attenuated by 14,15-EEZE, and those of 8,9-*trans*-EET were enhanced, suggesting an EET receptor-independent mechanism for 8,9-*trans*-EET that is amplified with 14,15-EEZE.

A role for *cis*-EETs in BP reduction in the SHR with sEH inhibition was not directly supported by this study of circulatory EETs; however, an elevation in both *cis*- and *trans*-EETs in tissues such as the kidney that are involved in BP regulation cannot be discounted. Imig et al. (24) reported decreased urinary EET excretion in rats with ANG II-induced hypertension and elevated excretion of 14,15-DHET, consistent with increased activity of sEH. Inhibition of sEH increased urinary EETs, decreased 14,15-DHET, and resulted in a diuresis that paralleled the fall in BP (24). In contrast to those results, we were unable to discern any effect of AUCB treatment of SHR and WKY rats for 7 days on the urinary excretion of EETs or

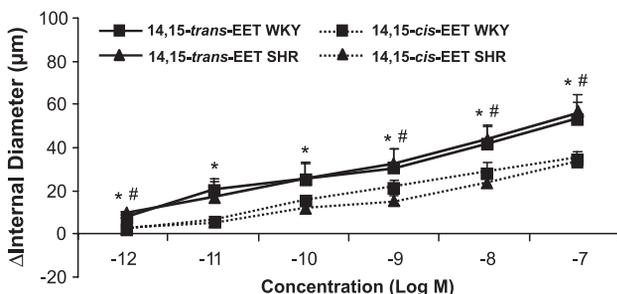


Fig. 4. Comparison of the vasoactivity of *cis*- and *trans*-EETs on pressurized rat arcuate arteries of 12-wk-old SHR and WKY rats. Arteries were pressurized to 80 mmHg and precontracted with phenylephrine, and concentration-response curves to 14,15-*cis*- or *trans*-EET were performed. * $P < 0.05$, 14,15-*trans*-EET compared with responses to 14,15-*cis*-EET on WKY arcuate arteries, $n = 4-5$. # $P < 0.05$, 14,15-*trans*-EET compared with responses to 14,15-*cis*-EET on SHR arcuate arteries, $n = 4-5$.

DHETs, possibly signifying a lack of effect of AUCB to inhibit sEH in our study. This possibility was highly unlikely, since RBCs obtained from rats treated with AUCB showed a marked impairment in the transformation of 14,15-*cis*- and 14,15-*trans*-EET to 14,15-*threo*- and 14,15-*erythro*-DHET, respectively. Similar to the results of Imig et al. for the urinary excretion of EETs in the ANG II hypertensive rat, we found that excretion of urinary EETs in the SHR was ~50% less than that in the WKY; however, we saw no increase in DHET excretion. It should be noted that the urinary EETs and DHETs analyzed in this study represented total amounts measured by GC/MS, which cannot distinguish between individual isomers.

The results of the present study confirm the beneficial effects of sEH inhibitors that have been reported for a variety of pathological conditions, including hypertension (45, 49), but extend these findings to invoke a novel mechanism for the hypotensive effect of AUCB that we postulate is mediated by an increase in plasma *trans*-EETs. The consideration of a role for RBCs in vascular regulation derives from their ability to release and hydrolyze EETs (25, 27, 28). Because RBCs are a major source of sEH in the circulation, inhibition of sEH elevated plasma EETs. This reflects a substantial increase in *trans*-EETs with little or no change in *cis*-EETs and is consistent with the preferred hydrolysis of *trans*- over *cis*-EETs by the sEH (29). Increased plasma levels of *trans*-EETs and greater vasodilator activity of *trans*- than *cis*-EETs may account for the reduction in BP by sEH inhibition in the SHR. Because activity of many CYP isoforms can be driven by lipid hydroperoxides (4, 40), formation of *cis*- and *trans*-EETs should be increased with enhanced endothelial expression of CYP epoxygenases, resulting in BP reduction (32). Thus, increasing CYP metabolism that consumes lipid hydroperoxides and forms *trans*-EETs may be a significant antioxidant and antihypertensive remedy (4, 7, 52).

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DISCLOSURES

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