Soluble epoxide hydrolase in the generation and maintenance of high blood pressure in spontaneously hypertensive rats

Maarten P. Koeners, Sebastiaan Wesseling, Arzu Ulu, Rocío López Sepúlveda, Christophe Morisseau, Branko Braam, Bruce D. Hammock and Jaap A. Joles

Am J Physiol Endocrinol Metab 300:E691-E698, 2011. First published 25 January 2011; doi:10.1152/ajpendo.00710.2010

Am J Physiol Endocrinol Metab 300: E691–E698, 2011.
First published January 25, 2011; doi:10.1152/ajpendo.00710.2010.

Soluble epoxide hydrolase in the generation and maintenance of high blood pressure in spontaneously hypertensive rats

Maarten P. Koeners,[1]* Sebastiaan Wesseling,[1]* Arzu Ulu,[3]* Rocío López Sepúlveda,[1,4] Christophe Morisseau,[3] Branko Braam,[1,2] Bruce D. Hammock,[3] and Jaap A. Joles[1]

[1]*Department of Nephrology and Hypertension, University Medical Center, Utrecht, The Netherlands;* [2]*Division of Nephrology and Immunology, Departments of Medicine, and Physiology, Edmonton, Alberta, Canada;* [3]*Department of Entomology and Cancer Center, University of California, Davis, Davis, California; and* [4]*Department of Pharmacology, School of Pharmacy, University of Granada, Granada, Spain*

Submitted 30 December 2010; accepted in final form 24 January 2011

Koeners MP, Wesseling S, Ulu A, Sepúlveda RL, Morisseau C, Braam B, Hammock BD, Joles JA. Soluble epoxide hydrolase in the generation and maintenance of high blood pressure in spontaneously hypertensive rats. *Am J Physiol Endocrinol Metab* 300: E691–E698, 2011. First published January 25, 2011; doi:10.1152/ajpendo.00710.2010.—We hypothesized that perinatal inhibition of soluble epoxide hydrolase (SEH), which metabolizes epoxyeicosatrienoic acids in the arachidonic acid (AA) cascade, with an orally active SEH inhibitor, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA), would persistently reduce blood pressure (BP) in adult SHR despite discontinuation of AUDA at 4 wk of age. Renal cytoplasmic epoxide hydrolase-2 (Ephx2) gene expression was enhanced in SHR vs. WKY from 2 days to 24 wk. Effects of perinatal treatment with AUDA, supplied to SHR dams until 4 wk after birth, on BP in female and male offspring and renal oxylipin metabolome in female offspring were observed and contrasted to female SHR for direct effects of AUDA (8–12 wk). Briefly, inhibition of SEH was effective in persistently reducing BP in female SHR when applied during the perinatal phase. This was accompanied by marked increases in major renal AA epoxides and decreases in renal lipoxygenase products of AA. Early inhibition of SEH induced a delayed increase in renal 5-HETE at 24 wk, in contrast to a decrease at 2 wk. Inhibition of SEH in female SHR from 8 to 12 wk did not reduce BP but caused profound decreases in renal 15(*S*)-HETrE, LTB4, TBX2, 5-HETE, and 20-HETE and increases in TriHOMEs. In male SHR, BP reduction after perinatal AUDA was transient. Thus, Ephx2 transcription and SEH activity in early life may initiate mechanisms that eventually contribute to high BP in adult female SHR. However, programmed BP-lowering effects of perinatal SEH inhibition in female SHR cannot be simply explained by persistent reduction in renal SEH activity but rather by more complex and temporally dynamic interactions between the renal SEH, lipoxygenase, and cyclooxygenase pathways.

hypertension; soluble epoxide hydrolase inhibitors; perinatal treatment; developmental plasticity

EPOXYEICOSATRIENOIC ACIDS (EETs) are potent vasodilator agents that act as endothelial-derived hyperpolarizing factors operating through calcium-activated potassium channels (6). Soluble epoxide hydrolase (SEH) metabolizes EETs to less active dihydroxyeicosatrienoic acids (DHETs) (9, 10). Recently, an orally active SEH inhibitor, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA), was found to largely attenuate angiotensin II (ANG II)-induced hypertension in mice (13). Similarly, AUDA and another SEH inhibitor, 1-cyclohexyl-3-dodecylurea (CDU), both attenuated ANG II-induced hypertension in rats (11, 42). However, in male stroke-prone spontaneously hypertensive rats (SHR), AUDA failed to affect blood pressure (BP) at the dosage used, although it did reduce the size of cerebral infarcts (3), and CDU only had a transient antihypertensive effect in male SHR (41). Indeed, some potent SEH inhibitors with promising pharmacokinetics failed to reduce BP over a period of 8 h in adult SHR even when used at very high doses (31, 32).

In light of the findings in ANG II-induced hypertension in mice and rats, failure of SEH inhibition to lower BP in some experiments in SHR is remarkable, as it is well known that inhibition of the renin-angiotensin system has a marked antihypertensive effect in SHR (34). One potential explanation for this inconsistency is related to the polymorphisms in cytoplasmic epoxide hydrolase-2 (Ephx2) gene, which results in different haplotypes with low vs. high Ephx2 gene expression. Indeed, although upregulation of renal Ephx2 gene expression has been documented in SHR from the Charles River stock between 3 and 18 wk (3, 7, 20, 30), this was not found in SHR from several other suppliers, where there was also no relation between SEH protein abundance and BP (7, 20). However, besides the Ephx2 haplotypes, the developmental stage of the rats when SEH is inhibited may affect progression and hence attenuation of hypertension in SHR.

Hypertension in the SHR is multifactorial, and target organ damage develops only after many months. It remains unclear whether SEH plays a pathogenic role during initiation or early development of hypertension in SHR before development of target organ damage. Previously, we were able to persistently reduce high BP in SHR offspring, especially females, by perinatal treatment with antioxidants and vitamins or with agents that improve NO availability (14, 25). In addition, we found, using microarray, enhanced renal Ephx2 gene expression in female SHR from 2 days to 48 wk (38). The hypothesis of the present study is that perinatal SEH inhibition persistently reduces BP in adult female SHR despite discontinuation of SEH inhibition at an early age.

To this end, two questions were addressed. *1*) Does reduction of activity of SEH with the SEH inhibitor AUDA during the perinatal period persistently lower BP in SHR offspring, and more so in females than in males? *2*) Does inhibition of SEH in young female SHR inhibit development of hypertension?

* M. P. Koeners, S. Wesseling, and A. Ulu contributed equally to this study.
Address for reprint requests and other correspondence: J. A. Joles, Dept. of Nephrology and Hypertension F03.226, Univ. Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands (e-mail: j.a.joles@umcutrecht.nl).

METHODS

Animals. SHR (Harlan-Olac Oxon, UK) and Wistar-Kyoto rats (WKY; Harlan-Olac) were housed at 22°C, humidity 60%, and exposed to a 12:12-h light-dark cycle. They were fed regular chow (Special Diets Services, Witham, Essex, UK). Sentinel animals were housed under the same conditions and regularly monitored for infection by nematodes and pathogenic bacteria and antibodies for rodent viral pathogens (International Council for Laboratory Animal Science, Nijmegen, The Netherlands). The Utrecht University Board for Studies in Experimental Animals approved the protocol.

SHR and WKY were bred. Kidneys were isolated from female and male SHR and female and male WKY at 2 days and 2 wk and kidney cortex at 24 wk ($n = 8$, 6, and 8 for female SHR; $n = 7$, 6, and 10 for male SHR; $n = 6$, 8, and 5 for female WKY; $n = 8$, 6, and 8 for male WKY at these respective ages). Renal cortical gene expression of Ephx2 was analyzed.

Medication. AUDA [12-(3-adamantan-1-yl-ureido)-dodecanoic acid] is a potent [rat $IC_{50} = 11$ nM using CMNPC as substrate (36)] [Suppl. Fig. S1 (supplementary materials are found with the online version of this paper at the Journal website)] and selective SEH inhibitor. However, its high lipophilicity and melting point, low water solubility and rapid β-oxidation make careful formulation critical to obtain effective blood concentrations (Suppl. Fig. S1 and Suppl. Table S1). AUDA was dissolved at a dose of 25 mg/l in drinking water containing 2-hydroxypropyl-β-cyclodextrin ("dextrin"; 10 g/l; Sigma-Aldrich) that was sonicated for ~1 h (13). AUDA concentrations in drinking water, determined by LC-MS analysis in (+) ion mode (see supplemental information and Suppl. Table S2), were 29 ± 1 mg/l (mean ± SD, $n = 6$) in freshly prepared water and decreased to 20 ± 1 mg/l in prepared water after 6 days at room temperature ($n = 3$). Therefore, drinking water solutions were refreshed four times a week.

To verify uptake of AUDA, we measured urinary excretion of AUDA, and its primary β-oxidation metabolite 4-(3-adamantan-1-yl-ureido)-butanoic acid (AUBA) in eight female and seven male pups from two AUDA-treated litters by liquid-liquid extraction with ethyl acetate followed by LC-MS analysis (see supplementary information) and in the 12-wk-old females that had been treated with AUDA from 8 wk of age. Furthermore, at 2 wk, when the pups were still entirely dependent on maternal intake, we measured AUDA and AUBA in renal tissue homogenates of treated and control pups.

Protocol 1. From day 7 of gestation, SHR females and their female and male offspring until 4 wk of age received either dextrin (10 g/l drinking water) or the SEH inhibitor AUDA (25 mg/l). Systolic BP (SBP) was regularly measured using tail cuff starting at 4 wk. At a younger age, SBP measurement is not feasible. At 28–30 wk, mean arterial pressure (MAP) and renal hemodynamics were measured. Urine (24-h) was collected at 4, 8, and 20 wk.

Protocol 2. From age of 8 wk, female SHR were treated with the SEH inhibitor AUDA (25 mg/l, $n = 8$) or dextrin only (10 g/l, $n = 8$) for 4 wk. At the end of the treatment (12 wk), MAP and renal hemodynamics were measured. SBP was measured regularly. Urine (24-h) was collected before starting treatment and after 1 and 4 wk of treatment.

Measurements under anesthesia. MAP and renal function were measured under anesthesia as previously described (14, 25) (see supplemental data).

SEH activity assay. Kidney tissue of 28- to 30-wk-old female rats was homogenized in sodium phosphate buffer (100 mM, pH 7.4) with a protease inhibitor (PMSF, 1 mM), centrifuged at 10,000 *g* for 10 min, and the remaining supernatant was centrifuged at 100,000 *g* for 1 h to obtain the 100,000-*g* soluble fraction (cytosolic fraction). [^{3}H]*t*-DPPO (*trans*-diphenylpropene oxide) radioactive partition assays were performed to detect the SEH activity in these separated cytosolic fractions (19). A BCA protein assay was used to detect the protein concentrations to calculate the specific activity in the cytosolic fractions.

Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. Renal oxylipins of female SHR were extracted using solid-phase extraction (SPE) followed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-MS-MS) analysis (40). Briefly, 250 μl of renal homogenate was put onto a C_{18} SPE cartridge with an antioxidant and internal standard solution (surrogate solution) and then pulled through the cartridge under low vacuum. After the samples were bound to the cartridge, oxylipins were eluted by ethyl acetate and evaporated to dryness with a vacuum centrifuge. The samples were then reconstituted with 50 μl of internal standard solution and run on Q-TRAP and submitted for LC-MS analysis.

Gene expression study. Renal cortex was isolated, snap-frozen, and further processed for gene expression study. In *protocol 1*, the gene expression of Ephx2 was analyzed by quantitative PCR (qPCR) using Taqman Gene Expression Assays (Applied Biosystems, Foster City, CA) as previously described (38). Genes studied were Ephx2 (Rn00576023) and 18S (Hs99999901) as a housekeeping gene. Calculation of the gene expression is based on $2^{-\Delta\Delta C_T}$ with age-matched WKY as the calibrator group.

Statistics. Data are shown as means ± SE. One-way analysis of variance (ANOVA) was performed on terminal data. Two-way ANOVA was performed on longitudinal data. Skewed data were log-transformed to achieve normality and equal variance. Where appropriate, ANOVA was followed by a Student-Newman-Keuls post hoc test.

RESULTS

Age effects. The enhanced gene expression of Ephx2, previously observed in female SHR (38), was confirmed at all ages compared with age- and sex-matched WKY ($P < 0.001$; females Fig. 1*A*; males Suppl. Fig. S2). Moreover, in 29-wk female rats, SEH activity was much higher in SHR than in WKY ($P < 0.001$; Fig. 1*B*).

There were no significant effects of age on renal epoxides [epoxyoctadecenoic acid (EpOME) and EET] in female SHR (see Controls in Table 1). However, renal diols [dihydroxyoctadecenoic acid (DiHOME) and DHET] varied strongly with age in control SHR, showing a marked increase from 2 wk to 12 wk (all $P < 0.01$) and then reversion to low levels at 29 wk, which were similar to those seen at 2 wk. Consequently, renal epoxide-to-diol ratios (EPOME/DiHOME and EET/DHET) were markedly reduced at 12 wk ($P < 0.01$). Similarly 15(*S*)-HETrE (hydroxyeicosatrienoic acid) was increased at 12 wk ($P < 0.05$). However, 9,12,13-TriHOME (9,12,13-trihydroxyoctadec-10-enoic acid) was increased in 29-wk SHR ($P < 0.05$).

As expected, aging in female SHR was accompanied by marked increases in proinflammatory metabolites in the kidney (see Controls in Table 2). Renal prostanoids and leukotriene B4 (LTB4), approximately doubled from 2 wk to 12 wk ($P < 0.05$) and further increased in 29-wk SHR ($P < 0.05$ vs. 12 wk and $P < 0.01$ vs. 2 wk). 8-hydroxyeicosatetraenoic acid (8-HETE) also increased with age. Lipoxin A4 (LXA4), thromboxane B2 (TBX2), 5-HETE, and 20-HETE showed marked increases from 2 wk to 12 wk and then reverted to very low levels in 29-wk SHR.

Protocol 1: perinatal SEH inhibition in female and male SHR. From 2 wk before birth, pregnant SHR were treated with SEH inhibitor AUDA or dextrin alone in drinking water. This was continued until weaning when the pups were 4 wk. Some pups were killed at 2 wk, when the pups were still entirely dependent on maternal intake, to study direct effects of SEH

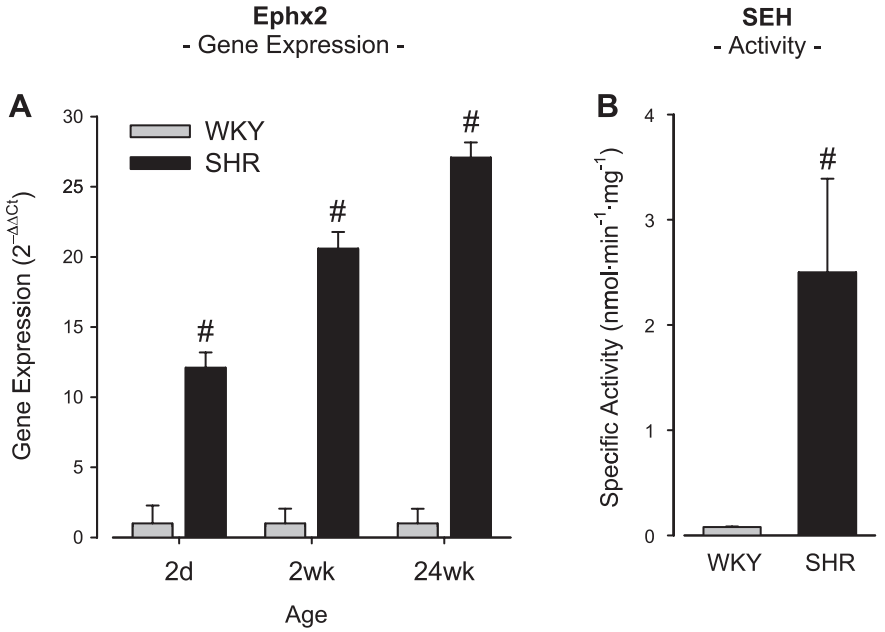

Fig. 1. Epoxide hydrolase-2 (Ephx2) gene expression and soluble epoxide hydrolase (SHE) enzyme activity in renal cortex of female spontaneously hypertensive (SHR) and Wistar-Kyoto rats (WKY) at different ages. Kidneys were isolated at 2 days and 2 wk and kidney cortex at 24 wk (n = 8, 6, and 8 for SHR; n = 6, 8, and 5 for WKY at these respective ages). *A*: SHR groups were compared with age-matched WKY as calibrator group for Ephx2 gene expression. *B*: specific activity of SEH in 28- to 30-wk SHR was compared with age-matched WKY. #$P < 0.01$ vs. WKY.

inhibition on renal AUDA and AUBA content (females and males) and renal oxylipins (only females). AUDA was <5 ng/ml in all samples, but kidneys of treated pups (females and males) contained considerable amounts of AUBA (379 ± 102 ng/ml), whereas AUBA was not detected in control kidneys. These data conclusively demonstrated that oral administration of AUDA via drinking water to the dams resulted in considerable intake in their pups.

At 2wk, there were no significant direct effects of SEH inhibition in female SHR on epoxides, but DiHOME were decreased by ~50% ($P < 0.05$; Table 1). Quantitatively, both 9(10)- and 12(13)-DiHOME contributed equally to this reduction (Suppl. Table S3). The DHETs also tended to decrease (Suppl. Table S3). However, overall, renal epoxide-to-diol ratios were not affected directly by SEH inhibition for either EPOME/DiHOME or EET/DHET (Table 1). In addition, 9,10,13-TriHOME and 15(*S*)-HETrE decreased in response to maternal SEH inhibition ($P < 0.05$; Table 1). At 2 wk, there were also no significant direct effects of SEH inhibition on renal prostanoids, TBX2, or 8-HETE and 20-HETE. However, renal 5-HETE and its metabolites LXA4 and LTB4 were reduced by more than 50% ($P < 0.05$; Table 2).

Although AUDA levels were below the detection limit (5 ng/ml) in all urine samples, treated pups excreted considerable amounts of AUBA at the end of treatment at 4 wk (females, 5,212 ± 1,467; males, 365 ± 150 ng·24 h^{-1}·100 g body wt^{-1}; Suppl. Fig. S2). By 8 wk (4 wk after stopping treatment), AUBA excretion was still detectable in all urine samples but had decreased by ~99% in females and males, respectively (23 ± 5 and 5 ± 1 ng·24 h^{-1}·100 g body wt^{-1} in females and males, respectively, $P < 0.001$ vs. 4 wk). Negligible amounts of AUBA could be measured at 20 wk in five samples in females (3 ± 2 ng·24 h^{-1}·100 g body wt^{-1}) and in seven samples in males (3 ± 1 ng·24 h^{-1}·100 g body wt^{-1}), but AUBA detection was undetectable (<9 ng/ml) in the other samples. Neither AUDA nor AUBA was detected in urine samples of control rats (n = 6: 3 females and 3 males) at any age. Note that although AUBA excretion at 4 wk was more

Table 1. *Renal EpOME, DiHOME, EET, DHET, TriHOME, and 15(S)-HETrE (μg/g tissue) and sum EpOME/DiHOME and EET/DHET ratios in 2-, 12-, and 29-wk (28- to 30-wk) control SHR or SHR with 4 wk of AUDA treatment at 2 wk (treatment from 2 wk before birth; AUDA) or 12 wk (treatment from 8 to 12 wk of age; AUDA) or at 29 wk (28–30 wk; treatment from 2 wk before birth to 4 wk of age; perinatal AUDA)*

	2 wk		12 wk		29 wk		2-Way ANOVA		
Age	Control	AUDA	Control	AUDA	Control	Perinatal AUDA	Age	AUDA	Interaction
n	4	5	3	5	8	9			
EpOME	36 ± 7	29 ± 2	26 ± 6	32 ± 8	37 ± 4	55 ± 5[b,d,f]	0.07	NS	(0.07)
DiHOME	21 ± 6	12 ± 3[e]	182 ± 13[b]	188 ± 31[b]	14 ± 2[d]	16 ± 1[d]	<0.001	NS	0.07
EpOME/DiHOME	2.1 ± 0.5	3.0 ± 0.55	0.15 ± 0.05[b]	0.16 ± 0.04[b]	2.8 ± 0.3[d]	3.7 ± 0.5[d]	<0.001	NS	NS
EET	186 ± 28	180 ± 8	169 ± 18	116 ± 17	209 ± 20	282 ± 22[b,d,f]	<0.001	NS	0.025
DHET	33 ± 7	23 ± 7	219 ± 40[b]	240 ± 54[b]	17 ± 2[a,d]	27 ± 4[d]	<0.001	NS	(0.07)
EET/DHET	6.6 ± 1.6	11.4 ± 3.3	0.84 ± 0.20[b]	0.56 ± 0.10[b]	13.2 ± 1.7[a,d]	12.2 ± 1.9[d]	<0.001	NS	NS
9,10,13-TriHOME	32 ± 4	16 ± 2[e]	28 ± 3	107 ± 9[b,f]	20 ± 2	28 ± 2[b,d]	<0.001	<0.001	<0.001
9,12,13-TriHOME	58 ± 7	29 ± 4	34 ± 5	124 ± 11[b,f]	153 ± 30[a,d]	86 ± 25[b,e]	0.002	NS	<0.001
15(*S*)-HETrE	56 ± 12	24 ± 5[e]	111 ± 16[a]	15 ± 9[f]	76 ± 17	42 ± 8[e]	NS	<0.001	0.029

Values are means ± SE. See text for definitions. [a]$P < 0.05$, [b]$P < 0.01$ vs. 2 wk; [c]$P < 0.05$, [d]$P < 0.01$ vs. 12 wk; [e]$P < 0.05$, [f]$P < 0.01$ vs. control.

Table 2. *Renal prostaglandins (PGE_2, PGD_2, 6-keto-PGF1a, PGJ_2), LXA4, LTB4, TBX2, and 5-, 8-, and 20-HETE (μg/g tissue) in 2-, 12-, and 29-wk (28–30 wk) control SHR or SHR with 4 wk of AUDA treatment either at 2 wk (treatment from 2 wk before birth; AUDA) or 12 wk (treatment from 8 to 12 wk of age; AUDA) or at 29 wk (28–30 wk; treatment from 2 wk before birth to 4 wk of age; perinatal AUDA)*

	2 wk		12 wk		29 wk		2-Way ANOVA		
Age	Control	AUDA	Control	AUDA	Control	Perinatal AUDA	Age	AUDA	Interaction
n	4	5	7	5	8	9			
PGE_2	25 ± 7	33 ± 9	71 ± 7[a]	70 ± 20	186 ± 41[b,f]	75 ± 32[f]	0.002	NS	0.031
PGD_2	11 ± 3	11 ± 3	40 ± 11[a]	46 ± 10[a]	147 ± 28[b,c]	40 ± 20[f]	<0.001	(0.06)	0.010
6-keto-PGF1a	17 ± 3	12 ± 1	26 ± 7	31 ± 7	119 ± 22[b,d]	44 ± 19[f]	0.004	(0.06)	0.049
PGJ_2	0.2 ± 0.1	0.2 ± 0.1	1.3 ± 0.4[b]	0.9 ± 0.1[a]	1.4 ± 0.2[b]	0.4 ± 0.2[d,f]	<0.001	0.030	0.009
LXA4	1.32 ± 0.20	0.51 ± 0.11[f]	3.2 ± 0.5[a]	1.86 ± 0.13[b]	0.49 ± 0.13[b,d]	0.83 ± 0.14[d]	<0.001	(0.07)	0.003
LTB4	2.7 ± 0.4	1.1 ± 0.1[e]	4.5 ± 0.7	0.9 ± 0.2[f]	8.7 ± 1.9[a,c]	3.2 ± 1.5[f]	0.006	<0.001	NS
TBX2	3.6 ± 0.6	3.7 ± 0.9	24 ± 2[b]	9.7 ± 0.6[b,f]	6.2 ± 0.7[a,d]	4.6 ± 0.4[d]	<0.001	0.002	0.037
5-HETE	124 ± 29	54 ± 12[e]	524 ± 68[b]	162 ± 10[b,f]	22 ± 7[b,d]	56 ± 10[d,f]	<0.001	(0.07)	<0.001
8-HETE	27 ± 3	14 ± 2	75 ± 12[b]	77 ± 6[b]	89 ± 23[a]	33 ± 10[a,d,f]	<0.001	0.006	0.048
20-HETE	21 ± 8	16 ± 4	130 ± 12[b]	64 ± 16[b,e]	10 ± 1[d]	19 ± 3[d,e]	<0.001	NS	0.005

Values are means ± SE. See text for definitions. [a]$P < 0.05$, [b]$P < 0.01$ vs. 2 wk; [c]$P < 0.05$, [d]$P < 0.01$ vs. 12 wk; [e]$P < 0.05$, [f]$P < 0.01$ vs. control.

than an order of magnitude higher in females than in males, by 20 wk, levels of AUBA excretion had decreased to similar trace levels.

Perinatal AUDA persistently reduced SBP in female SHR offspring from 8 wk onward (Fig. 2*A*) and in male SHR from 8 wk to 16 wk (Suppl. Fig. S4). Body weight was increased by perinatal AUDA from 8 wk in female and male SHR (Suppl. Fig. S5).

At the end of the experiment, the MAP measured under anesthesia at 28–30 wk was decreased by perinatal AUDA in adult female offspring ($P < 0.05$; Fig. 2*B*) but not in adult male offspring (Suppl. Fig. S4). There were no significant differences in kidney weight or fractional electrolyte excretions (Suppl. Tables S4 and S5), although GFR and ERPF were slightly lower in adult SHR males after perinatal AUDA. Twenty-four-hour protein excretion, which was very low in control adult SHR females, was even lower in adult SHR females exposed to perinatal AUDA ($P < 0.05$; Suppl. Table S4). Proteinuria was not affected by perinatal AUDA in adult SHR males (Suppl. Table S5).

Remarkably, perinatal treatment with AUDA (until weaning at 4 wk), although without any direct effects on renal epoxides at 2 wk, had marked incremental effects on renal epoxides at 29 wk (Table 1). Indeed EpOME, EET, and 9,10,13-TriHOME were ~50% higher in 29-wk rats perinatally treated with AUDA than in age-matched control rats, and, in contrast to control rats, there were also significant age-related increases for EpOME and EET. These changes were due to increases in all classes of EpOME and EET (Suppl. Table S3). Age-related increases vs. 2 wk were also seen for the TriHOMEs. Thus, regarding effects on renal epoxides, there was quite significant interaction between perinatal treatment with AUDA and aging. In contrast, perinatal AUDA had less overall effect on renal diols except for consistent decreases in 15(*S*)-HETrE (Table 1). However, several diols (Suppl. Table S3), as well as 9,10,13-TriHOME and 15(*S*)-HETrE (Table 1) showed interaction between age and AUDA effects (all $P < 0.05$). Overall renal epoxide-to-diol ratios were not affected in 29-wk SHR by perinatal SEH inhibition.

Similarly, perinatal treatment with AUDA, although without any direct effects on renal prostaglandins, TBX2, or 8-HETE at 2 wk, reduced these proinflammatory metabolites, often by more than 50%, at 24 wk (Table 2). Thus age-related proinflammatory effects on renal prostanoids LTB4, TBX2, and 8-HETE were partially reversed by perinatal treatment with AUDA, as illustrated by significant interaction ($P < 0.05$). In contrast, renal 5-HETE content was increased by ~50% ($P < 0.05$; Table 2), thus completely reversing the age-related re-

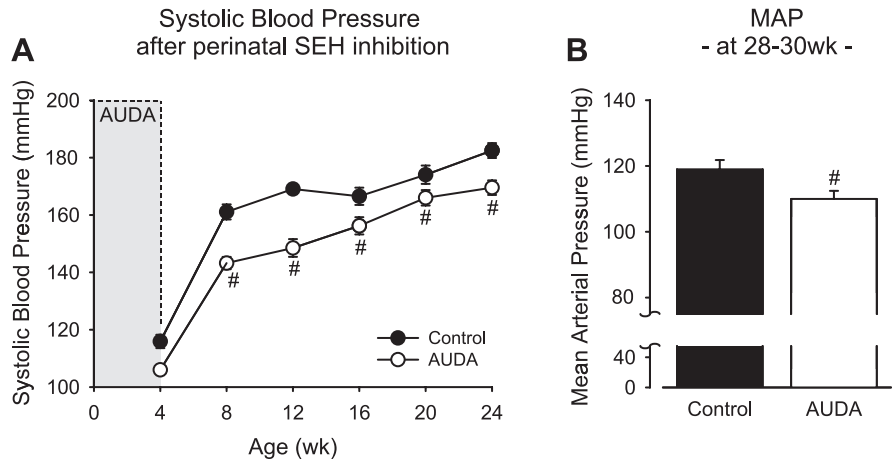

Fig. 2. Effect of perinatal SEH inhibition with AUDA [12-(3-adamantan-1-yl-ureido)-dodecanoic acid] on development of blood pressure in female SHR. Systolic blood pressure from 4 to 24 wk (*A*) and terminal mean arterial pressure (MAP) at 29 wk (*B*) in female SHR offspring. Control, $n = 18$ from 6 litters; perinatal AUDA, $n = 13$ from 5 litters. #$P < 0.05$ vs. SHR control.

duction. Thus, with respect to renal 5-HETE and 20-HETE content, direct and programmed effects of perinatal SEH inhibition were completely opposite, as was highlighted by very significant interaction ($P < 0.01$).

Protocol 2: SEH inhibition in female SHR from 8 to 12 wk. Because the antihypertensive effect of perinatal SEH inhibition with AUDA persisted up to 28 wk only in female SHR and the most marked antihypertensive effect in female SHR was observed during the developmental stage of hypertension from 8 to 12 wk of age, AUDA was supplied to female SHR from 8 wk of age for a period of 4 wk. At 12 wk, oral administration of AUDA also induced high levels of AUBA in the urine ($5{,}245 \pm 513$ ng·24 h^{-1}·100 g body wt^{-1}) of these female rats.

Remarkably, during the 4 wk of treatment, SBP was not significantly affected (Fig. 3*A*) despite intake of comparable amounts of AUDA as in 4-wk-old weanling female SHR (based on AUBA excretion; see Suppl. Fig. S2, *A* and *C*). After 4 wk of AUDA, there were also no effects on MAP (Fig. 3*B*), body and organ weights, renal hemodynamics, and 24-h protein excretion, except that the fractional excretion of sodium (FeNa) was increased in the AUDA-treated group ($P < 0.01$; Suppl. Table S6).

At 12 wk, there were also no significant direct effects of SEH inhibition on renal total epoxides or on DiHOME and DiHETErE (Table 1), although 12(13)-EPOME/DiHOME and 11(12)-EET/DHET ratios were decreased (both $P < 0.05$). Interestingly, AUDA induced marked direct increases of the TriHOMEs and decreased 15(*S*)-HETrE (all $P < 0.01$). There was also no direct effect of AUDA on renal prostaglandins. However, AUDA directly decreased LTB4, TBX2, 5-HETE, and 20-HETE but not 8-HETE (Table 2).

A simplified pathway of metabolites originating from arachidonic acid (AA) and a brief summary of the above-mentioned results are shown in Suppl. Fig. S6.

DISCUSSION

EETs are thought to have a stabilizing effect on BP. They reduce BP under conditions of hypertension (particularly in ANG II or DOCA models) and increase BP under conditions of hypotension. Thus the SEH inhibitors, which preserve EETs, generally have the same effects. The SHR rat is a complex model due to genetic diversity in general and increased expression of Ephx2 in particular. Indeed, inhibiting SEH in young female SHR with AUDA from 8 to 12 wk of age did not affect the development of hypertension in the present study. In contrast, after inhibiting SEH during the perinatal phase, the female offspring showed persistently reduced BP compared with untreated SHR. This effect was therefore sustained long after the SEH inhibitor had been cleared from the system. Although inhibiting SEH had few direct effects on renal epoxides, diols, or prostanoids, there were many long-term programmed effects such as marked increases in EpOME and EET, and equally profound decreases in renal prostaglandins, LTB4, and 8-HETE. Whether these changes are cause or consequence of the ameliorated hypertension is discussed below. Certainly these changes in regulatory lipids could be involved in the protection of kidneys and other organs. Apparently, programmed effects of perinatal treatment on BP in SHR cannot be simply explained by persisting changes in anatomic structures or functional pathways.

Initially, we documented a strong life-long increase in Ephx2 gene expression in SHR by use of independent techniques: microarray (38) and real-time PCR. Upregulation of renal Ephx2 gene expression has been documented in SHR derived from the Charles River stock between 3 and 18 wk of age (3, 7, 20, 30); however, this was not found in SHR from other suppliers (7, 20). We have been able to persistently reduce BP by several different perinatal treatments in SHR that came from the Harlan-Olac stock (14, 25). In this strain, we currently investigated renal Ephx2 gene expression and showed that female and male SHR from Harlan-Olac also have strongly increased Ephx2 gene expression compared with normotensive WKY (Harlan-Olac) from birth to old age. This encouraged us to continue the study of SEH inhibition in SHR with these Harlan-Olac strains.

Starting SEH inhibition 2 wk before to 4 wk after birth persistently reduced BP (~15 mmHg) in female SHR up to 29 wk of age. This novel finding suggests that SEH plays an important role in the initiation and maintenance of hypertension in female SHR. However, in male SHR perinatally treated with AUDA, although BP was initially and similarly reduced from 8 to 12 wk, BP was no longer reduced from 16 wk onward, and there was also no reduction in directly measured MAP at 29 wk. This sex difference was not unexpected, because previously we had also observed less pronounced antihypertensive effects of perinatal treatment in male than in female SHR (14, 25). Unexpectedly, AUBA excretion was much higher at 4 wk in AUDA-treated females than in males.

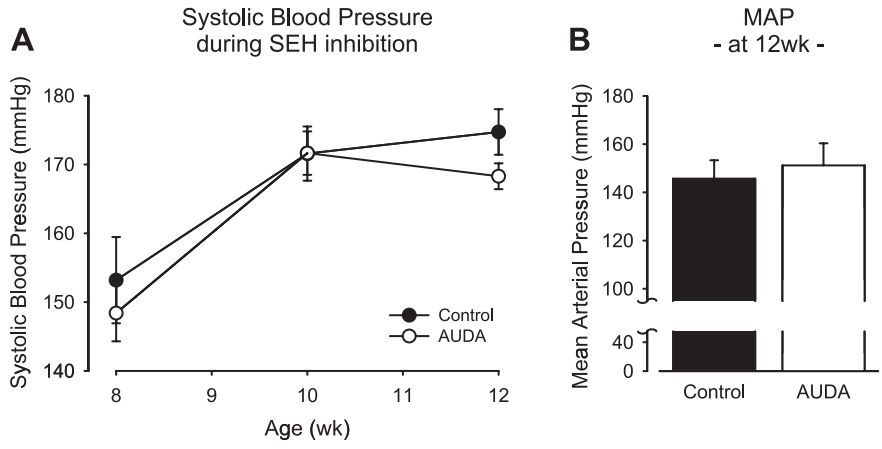

Fig. 3. Blood pressure development during SEH inhibition with AUDA from 8 to 12 wk of age in female SHR. Control, $n = 8$; AUDA, $n = 8$. *A*: systolic blood pressure; *B*: terminal MAP.

We have no explanation for this because body weights were already 10% higher at that age in males, suggesting normal intake of milk in the male pups. Conceivably, there are sex-related differences in compartmentalization or metabolism of AUDA at this early age. Note, however, that tissue levels at 2 wk of age, measured in both males and females, were quite high. Importantly, by 20 wk, AUBA excretion had fallen to negligible levels in females and males, but BP was still reduced in females but not in males. All in all, we conclude that, after perinatal administration of AUDA up to 4 wk, persistent effects observed for up to 6 mo after stopping treatment in adult rats do not depend on direct effects of AUDA or its metabolites.

Because the most profound reduction in BP in perinatally treated female SHR was observed between 8 and 12 wk of age, we tested whether inhibiting SEH in female SHR with AUDA for 4 wk, starting at 8 wk of age, would reduce BP. Strikingly, this was not the case. This unique constellation of findings in female SHR allows dissection of changes in the renal oxylipin metabolome directly associated with SEH inhibition from those associated with persistent BP reduction. At least three patterns emerge.

In female SHR, inhibiting SEH from 8 to 12 wk directly induced 12(13)-DiHOME, 9,10,13-TriHOME, and 9,12,13-TriHOME. These changes were clearly not related to any change in BP. In fact, in 29-wk-old rats with lower BP after perinatal SEH inhibition, 9,12,13-TriHOME was reduced. Similarly, inhibiting SEH in 8- to 12-wk SHR directly reduced 8(9)-EET, 5-HETE, and 20-HETE, whereas in 29-wk rats with lower BP after perinatal SEH inhibition, all three are increased. These contrasting patterns strongly suggest that persistent effects of perinatal SEH inhibition on BP in 29-wk SHR are not due to persistent changes in renal oxylipins. In a previous microarray study in SHR, we also failed to identify persistent changes in differential gene expression during and after perinatal treatment with dietary micronutrients despite persistent decrements in BP (38), underscoring the notion that persistent changes in functional pathways need not underlie persistent decreases in BP after perinatal treatment. Other mechanisms must be sought.

Direct effects of SEH inhibition on the renal oxylipin metabolome in 2-wk SHR included reductions in 12(13)-DiHOME, 5(6)-DHET, 14(15)-DHET, 9,10,13-TriHOME, 15(*S*)-HETrE, LXA4, LTB4, and 5-HETE. Of these, only 15(*S*)-HETrE and LTB4 were also reduced in 29-wk rats, with lower BP after perinatal SEH inhibition. Thus actions of 15(*S*)-HETrE and LTB4 in the juvenile kidney of SHR may indeed be important for long-term BP control. Although no information is available on the presence or action of 15(*S*)-HETrE in the kidney, our study suggests that this oxylipin may be viewed as a candidate factor for the development of hypertension. Leukotriene B4 and 5-HETE are well-known proinflammatory end products of the 5-lipoxygenase pathway (23, 28). Interestingly, although both were directly reduced by SEH inhibition in kidneys of 2-wk and 12-wk SHR, after perinatal SEH inhibition the levels in 29-wk SHR kidneys were divergent: LTB4 remained low, while 5-HETE levels increased, suggesting resetting of the 5-lipoxygenase pathway by perinatal SEH inhibition. Recently, we studied the plasma oxylipin metabolome in two conditions associated with inflammation: myocardial infarction and sepsis (17, 18). Both models revealed dynamic communication between the SEH, lipoxygenase, and cyclooxygenase pathways. The current study reveals that, during the development of hypertension (12), such dynamic communication also exists in the kidney of SHR. Renal inflammation is known to be an early event in SHR, an increased number of inflammatory cells already being observed at 3 wk of age (1, 26, 27). To our knowledge, the present study is the first in which the renal oxylipin metabolome has been studied in the different developmental phases of hypertension.

Finally, a host of changes occurred in the renal oxylipin metabolome of 29-wk female SHR with lower BP after perinatal SEH inhibition. Many of these can be viewed as being secondary to BP reduction, such as increases in some EpOMEs and EETs, 9,12,13-TriHOME, and 20-HETE, and decreases in several prostaglandins (PGE_2, PGD_2, 6-keto-PGF1α, PGJ2) and 8-HETE. Decreases in renal prostaglandins and urinary prostaglandin excretion often accompany BP reduction in other rodent models of hypertension (2, 4, 29) and are probably not specific for SEH inhibition. Indeed, in 12-wk SHR without BP reduction, SEH inhibition increased 9,12,13-TriHOME, quite the reverse effect. Opposite effects of SEH inhibition and BP reduction were also observed for 20-HETE that was decreased in 12-wk SHR after SEH inhibition and increased in 29-wk SHR after perinatal inhibition. Such differences also emphasize that the programming effects of perinatal SEH inhibition are not simply due to persistent reductions of SEH activity. However, exactly how long-term resetting of BP control occurs by perinatal SEH inhibition remains elusive.

A remarkable observation was the very high level of several oxylipins in young female SHR at 12-wk. This included renal diols (DiHOME and DHET) as well as LXA4, TBX2, 5-HETE, and 20-HETE. The pathophysiology of hypertension consists of three phases: early development (initiation), development, and maintenance. In female SHR, BP increases up to 12 to 16 wk and then stabilizes, whereas in male SHR, BP increases up to ~20 wk (14, 25). In the developing male SHR kidney, the production rates of DHETs were persistently increased severalfold vs. WKY from 3 to 13 wk (22, 41). This increase diminished with age, as was also found in the present study in female SHR kidney. Renal CYP4A2 mRNA levels and 20-HETE formation increase more in young SHR than in young WKY (15), and multiple CYP enzymes fall in their activity in SHR from 9 to 13 wk and stay down at 20 wk (21). Moreover, drugs that inhibit 20-HETE formation reduce BP in young SHR (16, 35, 37, 39). Similarly, drugs that inhibit thromboxane synthase ameliorate the increase in BP in young SHR (8, 24). Thus, high levels of these renal oxylipins in young SHR probably contribute to the steady increase in BP. Unfortunately the efficacy of such drugs is rarely tested in hypertensive rats older than 3 mo.

Hypertensive actions of *P*-450-dependent AA metabolites in the development of hypertension in SHR are well known (21). Thus the age-related increases in controls were not unexpected. Interestingly, long-term effects of perinatal SEH inhibition partially reversed most of these changes in conjunction with a reduction in BP. Whether these changes in renal prostanoids are the cause or the consequence of changes in BP is uncertain; however, programming effects on this axis have been recognized previously. Hypertensive effects of maternal low-protein diet are associated with increased renal PGE_2 up to 12 wk (33), in concordance with the reduction of renal PGE_2 and antihy-

pertensive effect after perinatal AUDA that we observed in 29-wk SHR. AUDA, one of the first SEH inhibitors developed, also is a weak PPARα agonist (5). It is unlikely that the levels of AUDA present in these experiments will reach concentrations needed to act as a PPAR agonist.

SHR perinatally exposed to AUDA demonstrated persistent growth enhancement in direct contrast to SHR perinatally exposed to a mixture of arginine, taurine, and vitamins C and E (ATCE), where growth was impeded (25). Considering that both perinatal ATCE and perinatal AUDA had long-term antihypertensive effects in female SHR, this clearly dissociates growth effects from changes in BP in this paradigm. Urinary protein excretion was slightly reduced in adult female SHR after perinatal AUDA. Whether this confers any long-term benefit is unknown.

In conclusion, Ephx2 gene transcription was increased throughout the first year of life in Harlan-Olac SHR compared with WKY. Despite this, only temporary inhibition of SEH with AUDA during the perinatal phase was effective in persistently reducing BP in female SHR. Thus, SEH may contribute to the initiation of mechanisms that eventually result in high BP in adult female SHR.

ACKNOWLEDGMENTS

We acknowledge Dr. Jun Yang for technical help on the LC-MS method.

GRANTS

The Dutch Kidney Foundation supported this study. Support was also provided by R37 ES-02710 and P42 ES-004699 from National Institute of Environmental Health Sciences and R01 HL-59699-06A1 from National Institutes of Health/National Heart, Lung, and Blood Institute. B. D. Hammock is a George and Judy Marcus senior scholar of the American Asthma Foundation. B. Braam is a New Investigator of the Heart and Stroke Foundation of Canada.

DISCLOSURES

No conflicts of interest are reported by the authors.

REFERENCES

1. **Biswas SK, de Faria JB.** Which comes first: renal inflammation or oxidative stress in spontaneously hypertensive rats? *Free Radic Res* 41: 216–224, 2007.
2. **Bivol LM, Hultstrom M, Gudbrandsen OA, Berge RK, Iversen BM.** Tetradecylthioacetic acid downregulates cyclooxygenase 2 in the renal cortex of two-kidney, one-clip hypertensive rats. *Am J Physiol Regul Integr Comp Physiol* 295: R1866–R1873, 2008.
3. **Dorrance AM, Rupp N, Pollock DM, Newman JW, Hammock BD, Imig JD.** An epoxide hydrolase inhibitor, 12-(3-adamantan-1-yl-ureido-)dodecanoic acid (AUDA), reduces ischemic cerebral infarct size in stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 46: 842–848, 2005.
4. **Duarte J, Perez-Palencia R, Vargas F, Ocete MA, Perez-Vizcaino F, Zarzuelo A, Tamargo J.** Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br J Pharmacol* 133: 117–124, 2001.
5. **Fang X, Hu S, Watanabe T, Weintraub NL, Snyder GD, Yao J, Liu Y, Shyy JY, Hammock BD, Spector AA.** Activation of peroxisome proliferator-activated receptor alpha by substituted urea-derived soluble epoxide hydrolase inhibitors. *J Pharmacol Exp Ther* 314: 260–270, 2005.
6. **Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R.** Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 401: 493–497, 1999.
7. **Fornage M, Hinojos CA, Nurowska BW, Boerwinkle E, Hammock BD, Morisseau CH, Doris PA.** Polymorphism in soluble epoxide hydrolase and blood pressure in spontaneously hypertensive rats. *Hypertension* 40: 485–490, 2002.
8. **Gomi T, Ikeda T, Ishimitsu T, Uehara Y.** Effects of OKY-046, a selective thromboxane synthetase inhibitor, on blood pressure and thromboxane synthesis in spontaneously hypertensive rats. *Prostaglandins Leukot Essent Fatty Acids* 37: 139–144, 1989.
9. **Imig JD.** Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases. *Am J Physiol Renal Physiol* 289: F496–F503, 2005.
10. **Imig JD, Navar LG, Roman RJ, Reddy KK, Falck JR.** Actions of epoxygenase metabolites on the preglomerular vasculature. *J Am Soc Nephrol* 7: 2364–2370, 1996.
11. **Imig JD, Zhao X, Zaharis CZ, Olearczyk JJ, Pollock DM, Newman JW, Kim IH, Watanabe T, Hammock BD.** An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* 46: 975–981, 2005.
12. **Johnson RJ, Rodriguez-Iturbe B, Kang DH, Feig DI, Herrera-Acosta J.** A unifying pathway for essential hypertension. *Am J Hypertens* 18: 431–440, 2005.
13. **Jung O, Brandes RP, Kim IH, Schweda F, Schmidt R, Hammock BD, Busse R, Fleming I.** Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension* 45: 759–765, 2005.
14. **Koeners MP, van Faassen EE, Wesseling S, de Sain-van der Velden M, Koomans HA, Braam B, Joles JA.** Maternal supplementation with citrulline increases renal nitric oxide in young spontaneously hypertensive rats and has long-term antihypertensive effects. *Hypertension* 50: 1077–1084, 2007.
15. **Kroetz DL, Huse LM, Thuresson A, Grillo MP.** Developmentally regulated expression of the CYP4A genes in the spontaneously hypertensive rat kidney. *Mol Pharmacol* 52: 362–372, 1997.
16. **Levere RD, Martasek P, Escalante B, Schwartzman ML, Abraham NG.** Effect of heme arginate administration on blood pressure in spontaneously hypertensive rats. *J Clin Invest* 86: 213–219, 1990.
17. **Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, Lu L, Yang J, Mochida H, Low R, Hammock BD, Chiamvimonvat N.** Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol* 47: 835–845, 2009.
18. **Liu JY, Yang J, Inceoglu B, Qiu H, Ulu A, Hwang SH, Chiamvimonvat N, Hammock BD.** Inhibition of soluble epoxide hydrolase enhances the anti-inflammatory effects of aspirin and 5-lipoxygenase activation protein inhibitor in a murine model. *Biochem Pharmacol* 79: 880–887, 2010.
19. **Morisseau C, Hammock BD.** Measurement of soluble epoxide hydrolase (sEH) activity. *Curr Protocol Toxicol* 33: 1–18., 2007.
20. **Okuda T, Sumiya T, Iwai N, Miyata T.** Difference of gene expression profiles in spontaneous hypertensive rats and Wistar-Kyoto rats from two sources. *Biochem Biophys Res Commun* 296: 537–543, 2002.
21. **Omata K, Abe K, Sheu HL, Yoshida K, Tsutsumi E, Yoshinaga K, Abraham NG, Laniado-Schwartzman M.** Roles of renal cytochrome P450-dependent arachidonic acid metabolites in hypertension. *Tohoku J Exp Med* 166: 93–106, 1992.
22. **Omata K, Abraham NG, Escalante B, Schwartzman ML.** Age-related changes in renal cytochrome *P*-450 arachidonic acid metabolism in spontaneously hypertensive rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 262: F8–F16, 1992.
23. **Osher E, Weisinger G, Limor R, Tordjman K, Stern N.** The 5 lipoxygenase system in the vasculature: emerging role in health and disease. *Mol Cell Endocrinol* 252: 201–206, 2006.
24. **Quest DW, Wilson TW.** Effects of ridogrel, a thromboxane synthase inhibitor and receptor antagonist, on blood pressure in the spontaneously hypertensive rat. *Jpn J Pharmacol* 78: 479–486, 1998.
25. **Racasan S, Braam B, van der Giezen DM, Goldschmeding R, Boer P, Koomans HA, Joles JA.** Perinatal L-arginine and antioxidant supplements reduce adult blood pressure in spontaneously hypertensive rats. *Hypertension* 44: 83–88, 2004.
26. **Rodriguez-Iturbe B, Quiroz Y, Ferrebuz A, Parra G, Vaziri ND.** Evolution of renal interstitial inflammation and NF-kappaB activation in spontaneously hypertensive rats. *Am J Nephrol* 24: 587–594, 2004.
27. **Rodriguez-Iturbe B, Zhan CD, Quiroz Y, Sindhu RK, Vaziri ND.** Antioxidant-rich diet relieves hypertension and reduces renal immune infiltration in spontaneously hypertensive rats. *Hypertension* 41: 341–346, 2003.
28. **Rubin P, Mollison KW.** Pharmacotherapy of diseases mediated by 5-lipoxygenase pathway eicosanoids. *Prostaglandins Other Lipid Mediat* 83: 188–197, 2007.
29. **Schnackenberg CG, Wilcox CS.** Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Iso prostaglandin f2alpha. *Hypertension* 33: 424–428, 1999.

30. **Seubert JM, Xu F, Graves JP, Collins JB, Sieber SO, Paules RS, Kroetz DL, Zeldin DC.** Differential renal gene expression in prehypertensive and hypertensive spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 289: F552–F561, 2005.
31. **Shen HC, Ding FX, Deng Q, Xu S, Chen HS, Tong X, Tong V, Zhang X, Chen Y, Zhou G, Pai LY, Alonso-Galicia M, Zhang B, Roy S, Tata JR, Berger JP, Colletti SL.** Discovery of 3,3-disubstituted piperidine-derived trisubstituted ureas as highly potent soluble epoxide hydrolase inhibitors. *Bioorg Med Chem Lett* 19: 5314–5320, 2009.
32. **Shen HC, Ding FX, Wang S, Xu S, Chen HS, Tong X, Tong V, Mitra K, Kumar S, Zhang X, Chen Y, Zhou G, Pai LY, Alonso-Galicia M, Chen X, Zhang B, Tata JR, Berger JP, Colletti SL.** Discovery of spirocyclic secondary amine-derived tertiary ureas as highly potent, selective and bioavailable soluble epoxide hydrolase inhibitors. *Bioorg Med Chem Lett* 19: 3398–3404, 2009.
33. **Sherman RC, Jackson AA, Langley-Evans SC.** Long-term modification of the excretion of prostaglandin E(2) by fetal exposure to a maternal low protein diet in the rat. *Ann Nutr Metab* 43: 98–106, 1999.
34. **Skov K, Fenger-Gron J, Mulvany MJ.** Effects of an angiotensin-converting enzyme inhibitor, a calcium antagonist, and an endothelin receptor antagonist on renal afferent arteriolar structure. *Hypertension* 28: 464–471, 1996.
35. **Su P, Kaushal KM, Kroetz DL.** Inhibition of renal arachidonic acid ω-hydroxylase activity with ABT reduces blood pressure in the SHR. *Am J Physiol Regul Integr Comp Physiol* 275: R426–R438, 1998.
36. **Tsai HJ, Hwang SH, Morisseau C, Yang J, Jones PD, Kasagami T, Kim IH, Hammock BD.** Pharmacokinetic screening of soluble epoxide hydrolase inhibitors in dogs. *Eur J Pharm Sci* 40: 222–238, 2010.
37. **Wang MH, Zhang F, Marji J, Zand BA, Nasjletti A, Laniado-Schwartzman M.** CYP4A1 antisense oligonucleotide reduces mesenteric vascular reactivity and blood pressure in SHR. *Am J Physiol Regul Integr Comp Physiol* 280: R255–R261, 2001.
38. **Wesseling S, Koeners MP, Kantouh F, Joles JA, Braam B.** Consequences of perinatal treatment with L-arginine and antioxidants for the renal transcriptome in spontaneously hypertensive rats. *Pflügers Arch* 458: 513–524, 2009.
39. **Xu F, Straub WO, Pak W, Su P, Maier KG, Yu M, Roman RJ, Ortiz De Montellano PR, Kroetz DL.** Antihypertensive effect of mechanism-based inhibition of renal arachidonic acid ω-hydroxylase activity. *Am J Physiol Regul Integr Comp Physiol* 283: R710–R720, 2002.
40. **Yang J, Schmelzer K, Georgi K, Hammock BD.** Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. *Anal Chem* 81: 8085–8093, 2009.
41. **Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, Kroetz DL.** Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res* 87: 992–998, 2000.
42. **Zhao X, Yamamoto T, Newman JW, Kim IH, Watanabe T, Hammock BD, Stewart J, Pollock JS, Pollock DM, Imig JD.** Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J Am Soc Nephrol* 15: 1244–1253, 2004.