Compensatory Mechanism for Homeostatic Blood Pressure Regulation in Ephx2 Gene-disrupted Mice

Ayala Luria, Steven M. Weldon, Alisa K. Kabcecnell, Richard H. Ingraham, Damian Matera, Huiping Jiang, Rajan Gill, Christophe Morisseau, John W. Newman, and Bruce D. Hammock

From the Departments of Entomology and Nutrition and the **Cancer Research Center, University of California, Davis, California 95616, the Departments of Translational Science and Cardiovascular Disease, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut 06877, and the United State Department of Agriculture, ARS, Western Human Nutrition Research Center, Davis, California 95616

Arachidonic acid-derived epoxides, epoxyeicosatrienoic acids, are important regulators of vascular homeostasis and inflammation, and therefore manipulation of their levels is a potentially useful pharmacological strategy. Soluble epoxyeicosatrienoic acid hydrolase converts epoxyeicosatrienoic acids to their corresponding diols, dihydroxyeicosatrienoic acids, modifying or eliminating the function of these oxylipins. To better understand the phenotypic impact of Ephx2 disruption, two independently derived colonies of soluble epoxide hydrolase-null mice were compared. We examined this genotype evaluating plasma levels of epoxy fatty acids, tissue oxylipin production capacity, and blood pressure. Ephx2 gene disruption eliminated soluble epoxide hydrolase protein expression and activity in liver, kidney, and heart from each colony. Plasma levels of epoxy fatty acids were increased, and fatty acid diols levels were decreased, while measured levels of lipoxygenase- and cyclooxygenase-dependent oxylipins were unchanged. Liver and kidney homogenates also show elevated oxylipin fatty acids. However, in whole kidney homogenate a 4-fold increase in the formation of 20-hydroxyeicosatetraenoic acid was measured along with a 3-fold increase in lipoxygenase-derived hydroxylation and prostanooid production. Unlike previous reports, however, neither Ephx2-null colony showed alterations in basal blood pressure. Finally, the soluble epoxide hydrolase-null mice show a survival advantage following acute systemic inflammation. The data suggest that blood pressure homeostasis may be achieved by increasing production of the vasococontractor, 20-hydroxyeicosatetraenoic acid in the kidney of the Ephx2-null mice. This shift in renal metabolism is likely a metabolic compensation for the loss of the soluble epoxide hydrolase gene.

Soluble epoxide hydrolase (sEH) is a ubiquitous enzyme found in many tissues such as liver, kidney, heart, and ovary (1). sEH catalyzes the degradation of endogenous epoxy lipids such as epoxyeicosatrienoic acids (EETs) to their less active diols (dihydroxyeicosatrienoic acids, DHETs) and hence plays a critical role in the control of EET levels (2). These epoxy lipids are potent vasodilators, regulating cerebral and renal homodynamic and blood pressure (3–5). In addition, EETs inhibit platelet aggregation (6), promote fibrinolysis (7) and have anti-inflammatory properties (8, 9). Whereas deletion of the sEH gene, Ephx2, has been reported to reduce blood pressure in male mice (10), the inhibition of endogenous EET hydrolysis may provide pharmacological benefit in hypertension and acute inflammation (11).

The human Ephx2 gene encodes sEH and consists of 19 exons encoding 555 amino acids (12). There is 73% homology between the human and mouse sEH protein sequences (13), with 100% conservation in the catalytic residues (14). Each monomer of the homodimeric mouse sEH has two distinct domains (14, 15). The N-terminal domain exhibits phosphatase activity, and the C-terminal domain is responsible for the epoxide hydrolysis activities (16, 17). Whereas the endogenous role of the phosphatase domain has yet to be elucidated, lipophilic phosphates appear to be excellent substrates (17–19).

Extensive investigations in recent years have established a role for cytochrome P450 (CYP)-derived eicosanoids in the regulation of blood pressure (11). The CYP-derived eicosanoids are synthesized throughout the body but act in a paracrine and autocrine fashion to regulate cellular function. In particular, the kidney and vascular endothelium produce significant levels of CYP-derived eicosanoids with effects on renal tubular transport function and vascular reactivity. Altered levels of renal EETs and hydroxyeicosatetraenoic acids (HETEs) are associated with changes in blood pressure (20–22). Furthermore, CYP epoxygenase activity is increased in animal models of hypertension, including the spontaneously hypertensive rats.
(21) and a high salt diet (23). The elevation in EET biosynthesis in these models could be a consequence of elevated blood pressure and might represent a compensatory response of the animal to deleterious increases in blood pressure. Moreover, inhibition of epoxygenases by clotrimazole leads to salt-dependent hypertension (24) and an inability to increase P450 epoxygenase activity with excess dietary salt intake in Dahl salt-sensitive rats might contribute to the hypertensive phenotype of these animals (23). In addition, with the onset of obesity-related hypertension the renal expression of CYP epoxygenases declines, while CYP omega hydroxylase and cyclooxygenase 2 expression increase (25, 26). Therefore, it would appear that the interplay of epoxygenase, ω-hydroxylases, and cyclooxygenase in the kidney are important determinants of blood pressure control.

To expand on the phenotypic characterization of the Ephx2-null mouse, animals were obtained from two independently derived and housed colonies of mice. The two colonies were either derived at the National Institutes of Health (10), or by Lexicon Genetics Inc. (The Woodlands, Texas). The latter, was exclusively for Boehringer Ingelheim Pharmaceuticals Inc. (Ridgefield, CT). Previous studies have shown that attenuation of endogenous EETs hydrolysis via sEH inhibition is a potentially useful pharmacological strategy for blood pressure regulation (4, 9, 27). In this regard, our main goal for this study was to perform a broad profile of the Ephx2-null phenotype and subsequently to evaluate blood pressure regulation under resting state as well as stressed conditions. We hypothesized that elevated endogenous EETs would have an advantageous role in blood pressure homeostasis and protect the overall health of the animal under abnormal conditions in which blood pressure is compromised.

EXPERIMENTAL PROCEDURES

Animals and Genotypic Analysis—Mice on a 129X1/SvJ × C57BL/6 background with targeted disruption in the Ephx2 gene were obtained from Chris Sinal, Dalhousie University, Halifax, NS, Canada under a National Cancer Institute Material Transfer Agreement 1-16268-04 (10). These mice backcrossed onto a C57BL6 genetic background three generations prior to their genetic background. Once obtained, Ephx2-null homozygotes were paired for subsequent breeding. Mice were maintained on sterile normal rodent diet (PicoLab rodent 20 from LabDiet, Richmond, IN) and bottled water ad libitum. Mice at age of 12–20 weeks were used and matched wild type mice were purchased from Taconic Farms. These animals were housed and bred in the Department of Cardiovascular Disease at Boehringer Ingelheim Pharmaceuticals, Inc. and are referred to as the BI colony.

BI Colony Blood Pressure—All procedures were conducted in accordance with approved IACUC protocols. Mean blood pressure (BP) and heart rate (HR) were monitored continuously in conscious, unrestrained female (20 weeks old) and male (12–20 weeks old) wild type and sEH-null mice using DSI telemetry (St. Paul, MN). Mice were surgically implanted with DSI TA11PA-20 telemetry transmitters and allowed to recover for a minimum of 7 days prior to study. Following recovery, mice were singly housed in plastic boxes placed directly on top of the telemetry receiver. Mean BP and HR data were recorded for 60 s every 20 min for the duration of the monitoring period. Individual mean BP and HR data were averaged over a 12-h cycle.

NIH Colony Blood Pressure and LPS Challenge—All procedures were conducted in accordance with approved IACUC protocols. Mean systolic blood pressure (SBP) and HR were measured in conscious, restrained wild type and Ephx2-null mice, starting at 8 weeks of age. Systolic blood pressure was measured by tail cuff plethysmography using a BP-2000 blood pressure analysis system (Visitech Systems, Apex, NC). Mice (n = 4 per genotype) were trained for 6 days to become acclimated to the Visitech System. For SBP and HR, twenty measurements were recorded daily and the last ten measurements averaged for a given mouse.

Western Blot Analysis—The expression of sEH or CYP4A proteins were analyzed by homogenization of tissues as described earlier (29). Briefly, protein concentrations were determined using the Pierce bicinchoninic acid protein assay. Protein aliquots from each tissue were resolved on 10–12% sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose membranes, and immunoblotted with polyclonal antibodies against rat CYP4A (Chemicon International, Temecula, CA) followed by horseradish peroxidase-conjugated secondary antibody (1:10,000). β-Actin antibodies (1:10,000) were purchased from Sigma, followed by anti-mouse horseradish peroxidase-conjugated secondary antibody (1:10,000). Chemiluminescent detection using ECL reagent from Amersham Biosciences (Piscataway, NJ) was carried out on x-ray film.

sEH Activity Assay—Soluble epoxide hydrolase activity was performed as previously described (29). Briefly, homogenates were diluted with sodium phosphate (100 mM, pH 7.4; 0.1 mg/ml bovine serum albumin) and chilled (on ice) until assayed. Assays were initiated by adding 1 μl of 5 μM [2-3H]-trans-1,3-diphenylpropene oxide (3H-tDPPO; [S]final = 50 μM) in N,N'-dimethylformamide to 100 μl of tissue homogenate. Tubes were incubated at 30 °C for 5 min. Reactions were quenched with 60 μl of methanol and 200 μl of isoctane (31). Controls for interfering glutathione-transferase activity were prepared as described previously, but were quenched with 60 μl
Characterization of Soluble Epoxide Hydrolase Null Mice

of methanol and 200 μl of hexanol (31). A 40-μl aliquot of the aqueous phase was analyzed for the presence of the radioactive diol product by liquid scintillation counting. Reactions were performed in triplicate. Results are means ± S.D. of three separate experiments.

Sample Collection and Oxylipin Extraction—Blood was collected from phenobarbitol-anesthetized mice by cardiac puncture in EDTA-rinsed syringes. Each sample was immediately spun (10 min, 400 × g), and the plasma was separated and stored at −80 °C until analysis. Urine was collected from male Ephx2-null or wild type mice (n = 3) housed 24 h in metabolic chambers. Food and water were provided ad lib. Urine was collected into dry ice-chilled tubes containing butylated hydroxytoluene and EDTA at 0.2 mg and 10 mg of triphenylphosphine. Samples were stored at −80 °C until analysis. Oxylipins were extracted by solid phase extraction. Detailed protocols are provided as Supplemental Data under “Oxylipin extraction” (32).

Oxylipin Production Assay—Polyunsaturated fatty acid oxylipases were determined in 59 fractions using modifications of previously reported procedures (29). Fatty acid epoxygenase, ω-hydroxylase, mid-chain hydroxylase, and prostaglandin synthase activities were obtained simultaneously. Briefly, aliquots (2 mg of protein) were suspended in 100 μl of 0.1 m sodium phosphate buffer (pH 7.4) and incubated (30 °C, 30 min) with or without arachidonic acid (100 μm) in the presence of an NADPH regenerating system. Reactions were halted with methanol containing analytical surrogates, and samples were centrifuged to remove precipitated protein. Arachidonate oxidation products were quantified in the isolated supernatant by HPLC/MS/MS. In the absence of arachidonic acid, no oxidation products were detected. Each reaction was performed in duplicate and mean specific activities were expressed in fmol/mg protein.

Oxylipin Analysis—Epoxides, diols, alcohols, and ketones of linoleate and arachidonate were quantified in all samples. Thromboxanes and prostaglandins were also measured in plasma and tissue production assays, but not urine because of matrix-dependent ion suppression. These oxylipins were quantified by HPLC/MS/MS using internal standard methods (32). Detailed protocols including HPLC (supplemental Table S1) and mass spectroscopy (supplemental Table S2) parameters are provided as Supplemental Data. Plasma and urine epoxide and diol surrogate recoveries were acceptable in all samples (see supplemental Table S3). Recoveries of tetradeuterated 6-keto-PGF1α (d4–6-keto-PGF1α) were acceptable in all samples (see supplemental Table S3) and used to quantify 6-keto-PGF1α. Epoxide surrogate hydrolysis was less than 3% for all assays. Reagent blanks showed background levels below detection limit, and analytical replicates showed precision to be within 15% for greater than 80% of the analytes present at levels greater than ten times the method detection limits.

Statistics—Values are expressed as mean ± S.D. or S.E., as indicated. Data were analyzed by Student’s t test or ANOVA, as indicated under the figure legends. Values were considered significantly different if p < 0.05.

RESULTS

sEH Gene, Protein Expression, and Activity—The sEH gene was not detected in either colony of Ephx2-null mice (Fig. 1A). Data are shown for the NIH colony. Identical results were obtained with the BI colony (data not shown). Evaluation of sEH protein expression was performed on tissues from representative animals from each colony. Polyclonal antibodies highly selective to sEH recognized a Protein band of 62.5 kDa in liver, kidney, and heart of wild type sEH mice from the BI and NIH colonies (Fig. 1B), but not in Ephx2-null mice (Fig. 1B). sEH was also observed in the lungs, spleen, adipose, and testis (data not shown) as previously reported (1). The distribution of the sEH protein was in agreement with published data as previously summarized (33). The results from the sEH tDPPO hydroxylase activity assays are shown in Table 1. As expected based on the immunoblots, hydroxylase activity was observed in wild type, but not in Ephx2-null tissues. Notably, the sEH activity measured in heart was significantly different between the Ephx2+/+ mice from the two colonies (Table 1). sEH activity was ten times higher in heart extract from the BI colony. However, the rank order activity in both colonies was the same.

Blood Pressure—The initial description of the Ephx2-null mouse indicated a male-specific hypotensive phenotype (10). To assess the maintenance of this phenotype in the two new
TABLE 1
sEH specific activities in liver, kidney, and heart tissue homogenates from the wild-type and Ephx2-null mice

| Genotype | BI colony | NIH colony |
|----------|-----------|------------|
| Ephx2+/+ | Ephx2−/− | Ephx2+/+ | Ephx2−/− |
| Liver    | n mole diol produced × min⁻¹ × mg protein⁻¹ | 32 ± 2 | <0.1 |
|          |          | 18 ± 3 | <0.1 |
|          |          | 14 ± 2 | <0.1 |
| Kidney   |          | 38 ± 5 | <0.01 |
|          |          | 16 ± 3 | <0.01 |
|          |          | 1.4 ± 0.2 | <0.04 |

A. [Graph showing mean blood pressure (BP) and heart rate (HR) for Ephx2+/+ and Ephx2−/− mice in the BI colony.]

B. [Graph showing mean blood pressure (BP) and heart rate (HR) for Ephx2+/+ and Ephx2−/− mice in the NIH colony.]

FIGURE 2. Elimination of sEH protein expression fails to cause detectable alteration in blood pressure. A, mean blood pressure (BP) and heart rate (HR) were recorded via telemetry (12-h averages) in wild-type and Ephx2-null mice from the BI colony. B, mean systolic blood pressure (SBP) and HR were recorded by tail cuff measurements in the NIH colony. Results are presented as mean ± S.E. for: δ Ephx2+/+ (n = 9); δ Ephx2−/− (n = 12); δ Ephx2+/− (n = 8) in the BI colony and mean ± S.E. for: δ Ephx2+/+ (n = 4) and δ Ephx2−/− (n = 4) in the NIH colony. Significant differences are indicated by ANOVA with Tukey-Kramer for telemetry and single factor ANOVA for tail cuff plethysmography (p < 0.05).

Colony, blood pressure and heart rate measurements were performed in NIH colony by tail cuff and in the BI colony by telemetry. In the BI colony, no physiologically relevant differences in mean blood pressure were noted between genotypes (Fig. 2A). There was a significant difference in mean blood pressure noted between female and male Ephx2-null mice but only on the first day of the 6-day monitoring period. Female wild type mice exhibited a trend toward higher overall heart rate levels compared with male wild type and Ephx2-null mice (Fig. 2A). These results are inconsistent with data published previously (10). To further investigate a potential strain difference, blood pressure was also assessed for 10 days in male Ephx2-null mice from the NIH-derived UC Davis colony compared with wild type. The NIH colony also failed to reveal a consistent difference in systolic blood pressure measurements between Ephx2-null and wild type mice (Fig. 2B). Several transient differences were measured on day 4 and 6, but were variable and not sustained. Therefore, no appreciable differences in resting blood pressure were detected between wild type and Ephx2-null genotypes from either of the two independently derived colonies.

Plasma Oxylipins—Plasma CYP- and sEH-dependent oxylipin profiles from wild type and Ephx2-null animals from each colony are shown in Table 2 and described graphically in supplemental Fig. S1A. Compared with their wild type counterparts, both the BI and NIH colony Ephx2-null mice showed elevated plasma levels of linoleate epoxides (EpOMEs) and 14(15)-EET. Levels of the linoleate diol 12,13-dihydroxyoctadecanoic acid (12,13-DHOME) were reduced in these mice. Significant reductions in the 14,15-DHET were also observed in the BI colony Ephx2-null mice. Fig. 3 displays enzymatic substrate to product ratios of plasma oxylipins for wild type and Ephx2-null mice from the BI colony (see Ref. 34 for NIH-derived colony results). Whereas the CYP-epoxygenase/sEH-dependent epoxide-diol ratios were altered by Ephx2 disruption, the sEH-independent, 9-ODE to 9-oxo-ODE ratio was unaffected in the Ephx2-null mice. In addition, the magnitude of the difference between the sEH-dependent substrate to product ratios was correlated with the distance of the oxidized center from the carboxyl carbon (n = 6, r > 0.81, p < 0.05; Fig. 3, inset). This trend mimics the substrate selectivity of the soluble epoxide hydrolase, which preferentially hydrolyzes epoxy fatty acids with epoxides positioned far from the carboxyl carbon as summarized in Newman et al. (33). Changes observed in plasma levels of the measured lipoxygenase- and cyclooxygenase-dependent metabolites were less than 2-fold. The CYP4A metabolite 20-HETE was not detected in plasma extracts (limit of detection, 0.1 nm).

LPS Treatment—Acute inflammation induced by LPS results in hypotension in mice that can be blocked by prophylactic treatment with sEH inhibitors (23). Similarly, the Ephx2-null mice showed a reduced susceptibility to LPS-induced hypotension (Fig. 4). All Ephx2-null mice treated with LPS (10 mg/kg, i.p.) survived, while 18% of the wild type mice died within the first 24 h. Systolic blood pressure (Fig. 4) and heart rates were reduced by LPS exposure in both genotypes. Five hours post-LPS administration HR in Ephx2-null mice was 462 bpm, while the wild type mice became so hypotensive that the blood pressure and HR dropped below detectable limits (Fig. 4). Over the next 24–48 h, the HRs in Ephx2-null mice increased to 505 ± 49 and 622 ± 54 beeps per minute (bpm), respectively, whereas HRs in the wild types remained low, 437 ± 27 and 433 ± 21 bpm at 24 and 48 h, respectively. Thus, compared with wild type, survival and recovery from the severe, acute hypotensive response to LPS exposure was enhanced among the Ephx2-null
Characterization of Soluble Epoxide Hydrolase Null Mice

Table 2
Plasma CYP- and sEH-dependent oxylipin concentration (nm) determined in wild type (Ephx2+/+) and Ephx2-null (Ephx2−/−) mice from the BI and NIH colonies

Values shown are mean ± S.E. (n = 3 – 5 per group). Results for 20-HETE were below the 0.1 nm limit of detection and are not shown.

| Oxylipin                               | Ephx2+/+ (nm) | Ephx2−/− (nm) | Ephx2+/+ (nm) | Ephx2−/− (nm) |
|----------------------------------------|---------------|---------------|---------------|---------------|
| Epoxygenase-dependent metabolism       |               |               |               |               |
| 12(13)-EPOME                           | 18.1 ± 1.6    | 124.0 ± 11*   | 12.6 ± 0.3    | 77.9 ± 11.7*  |
| 9(10)-EPOME                           | 11.2 ± 2.4    | 47.9 ± 4.2*   | 6.3 ± 0.4     | 23.8 ± 0.5*   |
| 14(15)-EET                            | 1.7 ± 0.3     | 3.2 ± 0.2*    | 0.4 ± 0.2     | 2.6 ± 0.2*    |
| 11(12)-EET                            | 1.4 ± 0.3     | 1.7 ± 0.1     | 0.4 ± 0.1     | 2.5 ± 0.5     |
| 8(9)-EET                              | 4.1 ± 0.6     | 5.0 ± 0.9     | 0.4 ± 0.1     | 1.5 ± 0.7     |
| 5(6)-EET                              | 4.3 ± 1.1     | 3.2 ± 1.0     | 13.3 ± 2.1    | 34.7 ± 10.5   |
| Soluble epoxide hydrolase-dependent metabolism |           |               |               |               |
| 12,13-DHOME                            | 48.9 ± 5.1    | 11.3 ± 1.6*   | 16.16 ± 0.02  | 7.2 ± 0.7*    |
| 9,10-DHOME                             | 24.5 ± 2.5    | 24.5 ± 4.5    | 7.4 ± 0.6     | 81.1 ± 0.8    |
| 14,15-DHET                            | 1.4 ± 0.1     | 0.2 ± 0.1     | 0.6 ± 0.2     | 0.4 ± 0.1     |
| 11,12-DHET                            | 1.0 ± 0.2     | 0.5 ± 0.1     | 0.36 ± 0.04   | 0.5 ± 0.1     |
| 8,9-DHET                              | 1.4 ± 0.5     | 1.46 ± 0.57   | 4.9 ± 2.4     | 7.1 ± 1.2     |
| 5,6-DHET                              | 2.2 ± 0.3     | 1.65 ± 0.24   | 0.3 ± 0.2     | 0.4 ± 0.1     |

* p < 0.05 versus wild type (2-tailed t test).

FIGURE 3. Plasma oxylipin substrate:product ratios in the wild type and Ephx2-null mice. Plasma epoxidodiol ratios were elevated in Ephx2-null mice over the wild type in both the lineolate (EPOMEDHOME) and arachidonate series (EET:DHET). Conversely the sEH-independent 9-HODE:9-oxo-ODE ratio was unaffected in the Ephx2-null mice. The magnitude of the difference between sEH-dependent substrate:product ratios showed a strong correlation (p < 0.05, r² > 0.81; Pearsons correlation) with the distance of the oxidized center from the carboxyl carbon (inset box). Significant differences are indicated with an asterisk (*, p < 0.05; 2-tailed t test).

FIGURE 4. Elimination of sEH protein expression reduces hypotensive effects of LPS challenge in mice. Genotype-specific effects on blood pressure and heart rate in response to LPS challenge. The mean systolic blood pressure (SBP) and heart rate (HR) responses were recorded via tail-cuff in the NIH colony before and periodically after administration of LPS (10 mg/kg, i.p.). At 5 h post-LPS administration, the wild type blood pressure and heart rate were below the instrument detection limit (dashed line). Recovery from the acute hypotensive effect of LPS exposure was enhanced among the Ephx2-null mice compared with their wild type counterparts as demonstrated by the attenuated SBP response at both 24 and 48 h post-LPS (single factor ANOVA. *, p < 0.05; #, p < 0.1).

genotype as demonstrated by the attenuated SBP response and mortality rate observed at both 24 and 48 h post-LPS (Fig. 4).

Tissue Oxylipin Production Capacity—Arachidonic acid metabolism was evaluated in the liver and kidney of the wild type and Ephx2-null mice to investigate the potential for compensatory mechanisms in this mutant strain (Table 3). In this assay, the biochemical activity of series of polyunsaturated fatty acid oxides was determined in S9 fractions using arachidonic acid as a substrate with a NADPH regenerating system. While NADPH can inhibit lipoxigenase activity, it does not alter the relative product profile with fatty acids (35). Metabolite production rates of cyclooxygenase, lipoxigenase, and P450 enzymes are expressed by the concentration of their corresponding metabolites in Table 3 and described graphically in supplemental Fig. S1B.

Liver homogenates from Ephx2-null mice generally produced higher concentrations of EETs and reduced DHETs as compared with liver homogenates from their wild type counterparts (Table 3). Specifically the 14(15)- and 8(9)-EETs were elevated, whereas, the 14,15- and 11,12-DHETs were reduced. The low affinity sEH regioisomer-substrate, 5(6)-EET and its corresponding metabolite in Table 3 and described graphically in supplemental Fig. S1B.

Whereas lowered DHETs and elevated EET production was observed in Ephx2-null kidney homogenates, the α-hydroxyl-
Characterization of Soluble Epoxide Hydrolase Null Mice

TABLE 3

Rates of arachidonic acid metabolism determined in BI colony Ephx2 wild type and -null mouse liver and kidney homogenates

Results are the mean ± S.E. of a minimum of 4 measurements of tissue samples from three mice per group. Differences in means were determined with 2-tailed t tests.

|                    | Liver                        | Kidney                      |
|--------------------|------------------------------|-----------------------------|
|                    | Ephx2^{+/+}                  | Ephx2^{−/−}                 | Ephx2^{+/+}                  | Ephx2^{−/−}                 |
|                    | fmoles/min × mg protein⁻¹    | fmoles/min × mg protein⁻¹   | fmoles/min × mg protein⁻¹    | fmoles/min × mg protein⁻¹   |
| Epoxygenase metabolism |                             |                             |                             |                             |
| 14(15)-EET         | 20.6 ± 5.3                   | 170 ± 27ᵃ                   | <0.1                        | 22.4 ± 12                   |
| 11(12)-EET         | <0.1                         | <0.1                        | <0.1                        | 68.7 ± 25ᵃ                  |
| 8(9)-EET           | 10.9 ± 0.94                  | 93 ± 3.9ᵃ                   | <0.1                        | 8.77 ± 1.2                  |
| 5(6)-EET           | 22.8 ± 6.9                   | 5.68 ± 0.89                 | <0.1                        | <0.1                        |
| Soluble epoxide hydrolase metabolism |                             |                             |                             |                             |
| 14,15-DHET         | 205 ± 44                     | 23.4 ± 8ᵃ                   | 58.9 ± 2.6                  | 11 ± 7.6ᵃ                   |
| 11,12-DHET         | 223 ± 43                     | 43.4 ± 11ᵃ                  | 58.6 ± 5.4                  | 15.7 ± 8.5ᵃ                 |
| 8,9-DHET           | <0.1                         | <0.1                        | 35.7 ± 6.8                  | 6.9 ± 0.5ᵃ                  |
| 5,6-DHET           | 49.7 ± 8.6                   | 10.8 ± 0.77ᵃ                | 11.3 ± 3.4                  | 8.61 ± 0.7                  |
| ω-Hydroxylation metabolism |                             |                             |                             |                             |
| 20-HETE            | 297 ± 63                     | 331 ± 30                    | 194 ± 130                   | 827 ± 54ᵃ                   |
| Lipoxygenase metabolism |                             |                             |                             |                             |
| 15-HETE            | 41.8 ± 9.3                   | 35.8 ± 6.3                  | 269 ± 46                    | 508 ± 40ᵃ                   |
| 12-HETE            | 44 ± 30                      | 27.5 ± 8.4                  | 93.5 ± 23                   | 150 ± 14ᵃ                   |
| 11-HETE            | 43.5 ± 9.1                   | 26.5 ± 4.1                  | 104 ± 2.8                   | 186 ± 26ᵃ                   |
| 8-HETE             | 66.2 ± 14                    | 42.7 ± 7.2                  | 47.1 ± 12                   | 117 ± 3.4ᵃ                  |
| 5-HETE             | 21.7 ± 3                     | 20.1 ± 3.8                  | 115 ± 18                    | 306 ± 22ᵃ                   |
| Cyclooxygenase metabolism |                             |                             |                             |                             |
| 6-keto-PGF_{1α}    | 10.3 ± 0.83                  | 8.94 ± 1.6                  | 13 ± 3                      | 124 ± 32ᵃ                   |
| PGF_{1α}           | 6.56 ± 1.6                   | 5.93 ± 0.93                 | 63.9 ± 7.6                  | 330 ± 72ᵃ                   |
| PGE₂               | 4.19 ± 0.26                  | 4.54 ± 0.093                | 9.78 ± 1.2                  | 33 ± 7.5ᵃ                   |
| Autooxidation marker |                             |                             |                             |                             |
| 9-HETE             | 8.76 ± 1.1                   | 8.66 ± 2                    | NAᵃ                        | NAᵃ                        |

ᵃ p ≤ 0.05. ᵇ 0.1 = below the 0.1 nmol of detection.

ᵇ NA, not analyzed.

As metabolite, 20-HETE was enhanced 4-fold. Moreover, the lipoxygenase-dependent metabolites (mid-chain HETEs) and the cyclooxygenase-dependent prostaglandins PGE_{2α}, PGE_{2β}, and 6-keto-PGF_{1α} were also increased 2–10-fold in the Ephx2-null kidney homogenates, suggesting up-regulation of these enzymes (Table 3).

Renal Feedback—Considerable evidence has accumulated indicating that expression of CYP enzymes and the production of EETs and 20-HETE in the kidney and peripheral vasculature are strong regulators of peripheral vascular tone, renal function, and blood pressure. Cellular expression of CYP 4A was measured in liver and kidney tissue homogenates. CYP 4A protein expression was 4-fold higher in whole kidney tissue homogenates from the Ephx2-null mice compared with wild type, and depressed in liver tissue homogenates of both genotypes (Fig. 5).

Urine oxylipin analysis from Ephx2-null or wild type male mice from the BI colony is presented in Fig. 6 and Fig. S1C. Whereas concentrations of metabolites among the pooled samples were high, the relative abundance of excreted metabolites showed significant differences between genotypes. Notably, the 12(13)-EpOME and 20-HETE were higher in the Ephx2-null urine, whereas the 12,13-DHOME, 14,15-DHET, and 5,6-DHET were all lower in this genotype. The concentrations of EETs were close to the detection limit in five of the six analyzed samples, likely precluding determination of changes in excretion of these metabolites.

**DISCUSSION**

Adaptation to defects in signaling, including genetic disruptions, are natural responses that allow organisms to maintain homeostatic control of critical phenotypic characteristics (36, 37, 38). An inability to compensate for such changes can lead to dire consequences (39). The initial description of the NIH-de
Characterization of Soluble Epoxide Hydrolase Null Mice

A.

Urinary oxylipin analyses showed changes in the relative abundance of excreted linoleate (A) and arachidonate (B) metabolites. A and B show the mean ± S.E. percentage of each analyte with respect to the quantified lipids of the same carbon number. Differences were determined by two-tailed t-tests and differences are indicated at the **, *p < 0.05 and *p < 0.1 levels.

B.

Ephx2-null mice in a structurally specific manner (Fig. 3), consistent with the known substrate specificity of the sEH. Thus, the 12,13-EpOME:DHOME and 14,15-EET:DHET ratios were the most sensitive indicators of sEH activity in these rodents. Interestingly, the epoxy fatty acid degradation was not totally abolished in the Ephx2-null mice (Ref. 10 and Table 2), suggesting an alternative route for their hydrolysis.

The CYP ω-hydroxylase metabolite, 20-HETE, plays a major role in vasoconstriction and renin natriuretic mechanisms (44). Recent reports further strengthen this concept and substantiate the link between CYP4A, 20-HETE, and hypertension (45–47).

Finally, in this study we provide data supporting the hypothesis that the Ephx2-null genotype is associated with a phenotypic adaptive response in renal lipid metabolism. This change results in maintenance of normal basal blood pressure. One possible explanation for the discrepancy between the blood pressure observations made by Sinal et al. (10) and those of this study could be related to the genetic background of the Ephx2 null mice. For example, previous data from Sinal et al. were obtained on a different genetic background and after far fewer generations than either of the two strains reported here. In addition, the earlier observations reported only small changes in blood pressure, which could be missed because of pressure oscillations in the current work. These results support a self-regulating interaction between the epoxygenase and ω-hydroxylase pathways producing a fine-tuned control over blood pressure homeostasis in the mouse.
Characterization of Soluble Epoxide Hydrolase Null Mice

Acknowledgments—We thank C. Williams, D. Cooper, R. Sellati, Mary McFarland, and Lynn Pantages-Torok for technical assistance and Theresa Pedersen for critical review of the manuscript. National Institutes of Health Images is a public domain image processing and analysis program.

REFERENCES

1. Enayetallah, A. E., French, R. A., Thibodeau, M. S., and Grant, D. F. (2004) J. Histochem. Cytochem. 52, 447–454
2. Spector, A. A., Fang, X., Snyder, G. D., and Weintraub, N. L. (2004) Prog. Lipid Res. 43, 55–90
3. Imig, J. D., Navar, L. G., Roman, R. J., Reddy, K. K., and Falck, J. R. (1996) J. Am. Soc. Nephrol. 7, 2364–2370
4. Imig, J. D., Zhao, X., Capdevila, J. H., Morisseau, C., and Hammock, B. D. (2002) Hypertension 39, 690–694
5. Harder, D. R., Zhang, C., and Gebremedhin, D. (2002) News Physiol. Sci. 17, 27–31
6. Heizer, M. L., McKinney, J. S., and Ellis, E. F. (1991) Stroke 22, 1389–1393
7. Node, K., Ruan, X. L., Dai, J., Yang, S. X., Graham, L., Zeldin, D. C., and Morisseau, C. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 2186–2191
8. Johnson, K. M., Renganarayanan, K., Frohlich, J. E., and Schiffrin, E. L. (2002) Circ. Res. 91, 1193–1199
9. Liao, J. K. (2001) Hypertension 37, 1563–1578
10. Sinal, C. J., Miyata, M., Tohkin, M., Nagata, K., Bend, J. R., and Gonzalez, J. W. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1640–1645
11. Seubert, J. M., Sinal, C. J., Graves, J., Degraff, L. M., Bradbury, J. A., Lee, C. R., Goralski, K., Carey, M. A., Lukia, A., Newman, J. W., Hammon, B. D., Falck, J. R., Roberts, H., Rockman, H. A., Murphy, E., and Zeldin, D. C. (2006) Circ. Res. 99, 442–450
12. Park, J. H., Cho, K., and Kim, S. (2002) Mol. Cell. Biol. 22, 4075–4084
13. Budayr, S. C., and Capdevila, J. H. (2003) Supp. Opin. Lipidol. 13, 273–283
14. Sandberg, M., and Meijer, J. (1996) Biochem. Biophys. Res. Commun. 221, 333–339
15. Beetham, J. K., Grant, D., Arand, M., Garbarino, J., Kiyosue, T., Pinot, F., Oesch, F., Belknap, W. R., Shinozaki, K., and Hammock, B. D. (1995) DNA Cell Biol. 14, 61–71
16. Argiriadi, M. A., Morisseau, C., Hammock, B. D., and Christianson, D. W. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10637–10642
17. Gomez, G. A., Morisseau, C., Hammock, B. D., and Christianson, D. W. (2004) Biochemistry 43, 4716–4723
18. Cronin, A., Mowbray, S., Durk, H., Homburg, S., Fleming, I., Fishtalber, B., Oesch, F., and Arand, M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1552–1557
19. Newman, J. W., Morisseau, C., Harris, T. R., and Hammock, B. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 1558–1563
20. Tran, K. L., Aronov, P. A., Tanaka, H., Newman, J. W., Hammock, B. D., and Morisseau, C. (2005) Biochemistry 44, 12179–12187
21. Enayetallah, A. E., and Grant, D. F. (2006) Biochem. Biophys. Res. Commun. 341, 254–260
22. Su, P., Kaushal, K. M., and Kroetz, D. L. (1998) Am. J. Physiol. 275, R426–R438
23. Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A. J., Newman, J. W., Parker, C., Graham, L., Engler, M. M., Hammock, B. D., Zeldin, D. C., and Kroetz, D. L. (2000) Circ. Res. 87, 992–998
24. Yu, Z., Straub, W. O., Pak, W., Su, P., Maier, K. G., Xu, M., Roman, R. J., Ortiz de Montellano, P. R., and Kroetz, D. L. (2002) Am. J. Physiol. Regul. Integ. Comp. Physiol. 283, R710–R720
25. Makita, K., Takahashi, K., Karara, A., Jacobson, H. R., Falck, J. R., and Capdevila, J. H. (1994) J. Clin. Investig. 94, 2414–2420
26. Holla, V. R., Adas, F., Imig, J. D., Zhao, X., Price, E., Jr., Olsen, N., Kovacs, W. J., Magnusson, M. A., Keeney, D. S., Breyer, M. D., Falck, J. R., Waterman, M. R., and Capdevila, J. H. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5211–5216
27. Imig, J. D., Falck, J. R., and Inscho, E. W. (1999) Br. J. Pharmacol. 127, 1399–1405
28. Messer-Letienne, L., Bernard, N., Roman, R. J., Sassard, J., and Benzoni, D. (1999) Eur. J. Pharmacol. 378, 291–297
29. Sarks, A., Lopez, B., and Roman, R. J. (2004) Curr. Opin. Nephrol. Hypertens. 13, 205–214