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This information is current as of September 29, 2009.
Soluble epoxide hydrolase gene deletion attenuates renal injury and inflammation with DOCA-salt hypertension

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Submitted 18 February 2009; accepted in final form 22 June 2009

Manhiani M, Quigley JE, Knight SF, Tasoobshirazi S, Moore T, Brands MW, Hammock BD, Imig JD. Soluble epoxide hydrolase gene deletion attenuates renal injury and inflammation with DOCA-salt hypertension. Am J Physiol Renal Physiol 297: F740–F748, 2009. First published June 24, 2009; doi:10.1152/ajprenal.00098.2009.—Inhibition of soluble epoxide hydrolase (sEH) has been shown to be renal protective in rat models of salt-sensitive hypertension. Here, we hypothesize that targeted disruption of the sEH gene (Ephx2) prevents both renal inflammation and injury in deoxycorticosterone acetate plus high salt (DOCA-salt) hypertensive mice. Mean arterial blood pressure (MAP) increased significantly in the DOCA-salt groups, and MAP was lower in Ephx2−/− DOCA-salt (129 ± 3 mmHg) compared with wild-type (WT) DOCA-salt (145 ± 2 mmHg) mice. Following 21 days of treatment, WT DOCA-salt urinary MCP-1 excretion increased from control and was attenuated in the Ephx2−/− DOCA-salt group. Macrophage infiltration was reduced in Ephx2−/− DOCA-salt compared with WT DOCA-salt mice. Albuminuria increased in WT DOCA-salt (278 ± 55 μg/day) compared with control (17 ± 1 μg/day) and was blunted in the Ephx2−/− DOCA-salt mice (97 ± 23 μg/day). Glomerular nephrin expression demonstrated an inverse relationship with albuminuria. Nephrin immunofluorescence was greater in the Ephx2−/− DOCA-salt group (3.4 ± 0.3 RFU) compared with WT DOCA-salt group (1.1 ± 0.07 RFU). Reduction in renal inflammation and injury was also seen in WT DOCA-salt mice treated with a SEH inhibitor [trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid; tAUCB], demonstrating that the C-terminal hydrolase domain of the sEH enzyme is responsible for renal protection with DOCA-salt hypertension. These data demonstrate that Ephx2 gene deletion decreases blood pressure, attenuates renal inflammation, and ameliorates glomerular injury in DOCA-salt hypertension.

deoxycorticosterone acetate; blood pressure; Ephx2; high salt; albuminuria; NF-κB; glomerular injury

EPoxyEicosatrienoic Acids (EETs), the products of cytochrome P-450 epoxygenase metabolism of arachidonic acid, have been shown to cause vasodilation, possess anti-inflammatory properties, prevent migration in vascular smooth muscle cells as well as prevent platelet aggregation (2, 11, 16, 39, 42). EETs cause vasodilation by activating large-conductance Ca2+-activated K+ channels in smooth muscle that leads to hyperpolarization and subsequent vasodilation (3, 10, 18). In addition, epoxygenase metabolites prevent the activation of NF-κB that in turn leads to the activation of downstream inflammatory cytokines (9, 38). EETs, however, are quickly degraded by soluble epoxide hydrolase (sEH) to their less active diols, resulting in diminished EET cardioprotective actions (16, 48, 50).

Past studies have demonstrated that pharmacological inhibition of sEH prevents EET degradation and enhances the renal and cardioprotective effects of these metabolites (11, 16, 17). Antihypertensive and renal-protective therapeutic actions for sEH inhibitors have been repeatedly demonstrated in angiotensin-dependent hypertension (19, 20, 21, 52). For instance, Zhao et al. (52) determined that hypertensive rats treated with an sEH inhibitor were protected against renal injury as seen by reduced collagen deposition and albuminuria. In addition to reduced renal injury with sEH inhibition, Cyp2c23, the key enzyme in the generation of EETs, has been shown to be upregulated when inflammatory pathways were inhibited (7, 8). For example, another study demonstrated that chemokine receptor 2b (CCR2b) inhibition enhanced renal Cyp2c23 expression in angiotensin-induced salt-sensitive hypertension (7). sEH inhibition has also been examined in non-angiotensin II models of hypertension and has been shown to lower blood pressure (29, 50). For instance, Loch et al. (29) demonstrated that sEH inhibition lowered blood pressure in rats treated with deoxycorticosterone acetate plus high salt (DOCA-salt).

Mice that are sEH null (Ephx2−/−) have been generated, and studies have begun to examine cardiovascular function and blood pressure regulation (30, 35, 45, 46, 51). Specifically, Ephx2−/− mice have been shown to have lower blood pressure as well as are protected against posts ischemic injury and ventricular dysfunction (35, 45, 46). The Ephx2 gene contains two domains: a hydrolase and a phosphatase domain (37). There are currently no known selective inhibitors of the N-terminal phosphatase domain that are active in vivo, and the sEH inhibitors inhibit the epoxide hydrolase activity of the C-terminal domain without affecting the phosphatase activity of the N-terminal domain (22, 33, 34). Definitive evidence that the phosphatase domain does not contribute to blood pressure regulation and renal damage in the Ephx2−/− mice is lacking. Accordingly, the hypothesis of the current study was that Ephx2 gene deletion reduces renal injury and inflammation with salt-sensitive hypertension and that this protection resides in the deficiency in the C-terminal hydrolase domain.

METHODS

Experimental hypertension groups. All animal studies were performed in accordance with the Medical College of Georgia Animal Care and Use Committee. Wild-type (WT) C57BL/6J mice (Jackson Laboratory, Sacramento, CA) and homozygous Ephx2 gene-deleted mice (Ephx2−/−) from Jackson Laboratories that were backcrossed with C57BL/6J mice for 10 generations were utilized. Adult WT and...
Ephx2−/− male mice weighing ~25 g were randomly assigned into four groups; control, high salt (1% NaCl drinking water), DOCA alone, and DOCA plus high salt (DOCA-salt). Mice were anesthetized with 2% isoflurane with a continuous flow of 95% O2-5% CO2. Mice were assigned to the DOCA alone and DOCA-salt groups. High salt was administered via drinking water containing 1% NaCl. Following 21 days of DOCA and/or high salt, mice were placed into metabolic cages, and a 24-h urine sample was collected. Animals were then anesthetized with pentobarbital sodium, and tissues were collected. All tissue samples were immediately frozen in liquid nitrogen and then stored at −80°C. Blood pressure and heart rate were measured using radiotelemetry. Mice were implanted with telemetry catheters as described previously (27).

Renal injury. An index of renal injury measured in the current study was albuminuria and was determined with an ELISA kit purchased from Exocell (Philadelphia, PA). Renal injury was further assessed histologically. Kidney sections embedded and frozen in Optimal Cutting Temperature (Tissue-Tek, Hatfield, PA) medium were sliced into 4-μm sections and stained with Masson’s trichrome for collagen III deposition according to the manufacturer’s recommended protocols (Richard Allan Scientific, Kalamazoo, MI). Ten images were taken per mouse, and values were averaged. To quantify the Masson’s trichrome staining, 10 random images each from all groups were assigned random numbers and scored by a blinded observer on a scale of 0 to 10 for collagen deposition. A score between 0 and 2 corresponded to intermediate staining, and 7–10 corresponded to high to very high staining intensity. Nephrin, a protein involved in the maintenance of the glomerular slit diaphragm, was also examined via immunofluorescence. Five-micrometer frozen kidney sections were incubated overnight at room temperature with goat anti-human nephrin primary antibody 1:50 (sc-19000, Santa Cruz Biotechnology) followed by rabbit anti-goat Cy-3 fluorescent-tagged secondary antibody 1:400 for 1 h (Zymed). Slides were mounted using Prolong Gold anti-fade (Invitrogen). Desmin immunofluorescence was carried out using a 1:50 dilution of mouse anti-human desmin primary antibody (Dako, Carpinteria, CA) followed by a 1:800 dilution of FITC-tagged goat anti-mouse secondary antibody (Zymed). Photographs were taken at ×400.

Renal inflammation. We determined whether mRNA expression of proinflammatory cytokines is altered in the DOCA-salt groups. Total RNA was extracted from 20 mg of kidney cortex using an RNeasy Plus Mini-kit (Qiagen) according to the manufacturer’s protocol. RNA concentrations were determined using absorbance at 260 nm. Reverse transcription was performed on 2 μg of RNA from each sample using an RT² PCR Array First Strand Kit (SuperArray Bioscience). Each cDNA synthesis reaction was diluted before being added as an RT² Real-Time SYBR Green PCR Mastermix (SuperArray Bioscience), which was aliquoted onto a 96-well PCR Array plate, one sample per plate; each well contained a primer pair for a different gene or control. Thermal cycling and real-time detection were done with a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, CA): 1) 95°C for 10 min and 2) 95°C for 15 s followed by 60°C for 60 s (repeated 40 times). Melt-curve analysis was completed after each PCR reaction. Threshold cycle (Ct) values were normalized to a set of housekeeping genes to get a ΔΔCt value, and fold-changes were calculated using the equation (2−ΔΔCt)×2−△△Ct control)−1. Student’s t-test was used for statistical analysis, and changes greater than ±2 and P < 0.05 were considered significant. Suparray results were confirmed by real-time PCR on three genes present on the arrays, picked at random.

Next, we examined macrophage infiltration into the kidney utilizing ED-1 immunohistochemistry. Five-micrometer frozen kidney sections were cut and incubated overnight at room temperature with mouse anti- rat CD-68 primary antibody (1:100, Serotec, Raleigh, NC) followed by the secondary antibody goat anti-mouse IgG HRP (1:50, Serotec) for 1 h at room temperature. Slides were incubated with AEC substrate chromogen (Dako) for 20 min, rinsed, and counterstained with Mayers hematoxylin for 30 s. Photographs were taken at ×400 magnification, and CD-68 positive cells were counted in a blinded fashion. The number of positive cells was calculated per square millimeter. To further assess cytokines and activation of inflammatory pathways, we measured MCP-1 and NF-κB activity. Urinary monocyte chemmatoactant protein-1 (MCP-1) excretion was measured using an ELISA purchased from BD Bioscience (MCP-1 ELISA kit, Minneapolis, MN). To assess activation of proinflammatory cytokines, an NF-κB activity assay was performed on kidney samples. Renal NF-κB activity was measured using a TransAM NFκBp65 activation assay purchased from Active Motif (Carlsbad, CA) and performed according to the manufacturer’s protocol.

Inhibitor studies. To determine that renal protection resides within the hydrolase domain of the Ephx2 gene, sEH inhibitor studies were performed in WT and Ephx2−/− DOCA-salt mice. These groups of mice were given the sEH inhibitor tAUCB at a dose of 10 mg/day in their food for 21 days. Blood pressure, albuminuria, and urinary MCP-1 excretion were examined following the 21-day treatment period using the methods explained previously.

Statistical analysis. All data are presented as means ± SE. For analysis of mean arterial pressure (MAP), statistical significance was determined using a two-way ANOVA followed by a Bonferroni post hoc test to identify individual differences between specific groups and treatment time. A P value of <0.05 was considered as statistically significant.

RESULTS

Blood pressure. MAP increased significantly from baseline in the WT DOCA-salt group at day 6 and remained increased for the remainder of the treatment period (Fig. 1A, *P < 0.05 vs. WT control). MAP also increased significantly in the Ephx2−/− DOCA-salt group compared with control at day 8 and remained elevated during the treatment period (+P < 0.05 vs. Ephx2−/− control). A separate set of WT and Ephx2−/− DOCA-salt mice were treated with tAUCB, a sEH inhibitor that selectively inhibits the hydrolase domain of the enzyme. MAP in the WT DOCA-salt group with tAUCB was significantly lower than WT DOCA-salt, and no statistical change was seen compared with Ephx2−/− DOCA-salt or Ephx2−/− DOCA-salt plus tAUCB (Fig. 1B). Heart rate decreased significantly in the WT DOCA-salt group with WT control (470 ± 12 vs. 576 ± 9 beats/min, P < 0.05) and also decreased in the Ephx2−/− DOCA-salt group compared with control (507 ± 15 vs. 572 ± 8 beats/min, P < 0.05). However, heart rate was significantly higher in the Ephx2−/− DOCA-salt group compared with WT DOCA-salt (P < 0.05).

To ensure that Ephx2−/− mice did not express sEH, Western blot analysis for sEH protein was performed on WT as well as Ephx2−/− kidney homogenates. Ephx2−/− mice did not express sEH protein (Fig. 1C). Since the Cyp2e44 protein is the major enzyme responsible for EET generation in the kidney of mice, Cyp2e44 expression was also examined in WT and Ephx2−/− mice kidneys, and expression remained similar in both groups (Fig. 1D).

Renal damage. Albuminuria was measured to assess renal damage (Fig. 2A). Albuminuria increased significantly in the WT DOCA-salt compared with WT control (+P < 0.05), and levels were attenuated in the Ephx2−/− DOCA-salt group (+P < 0.05 vs. WT DOCA-salt). Albuminuria was also reduced in the WT DOCA-salt plus tAUCB group compared with WT DOCA-salt (+P < 0.05). Renal collagen deposition
was assessed using Masson's trichrome staining (Fig. 2B). The intensity of Masson's trichrome stain increased significantly in the Ephx2−/− DOCA-salt group compared with the respective controls (n = 6/group *P < 0.05; A). MAP in the Ephx2−/− DOCA-salt group was significantly lower than WT DOCA-salt. MAP measurements were also made in WT and Ephx2−/− DOCA-salt groups treated with the soluble epoxide hydrolase (sEH) inhibitor tAUCB (n = 6/group; B). MAP was significantly lower in WT and Ephx2−/− DOCA-salt + tAUCB groups compared with WT DOCA-salt, and there is no difference in MAP between Ephx2−/− and WT DOCA-salt tAUCB-treated groups. sEH protein expression was absent from the Ephx2−/− mice (C), and Cyp2c44 expression remained unchanged in both WT and Ephx2−/− kidney homogenates (D).

Fig. 1. Mean arterial pressure (MAP) increased significantly in both wild-type (WT) and Ephx2−/− DOCA-salt groups compared with their respective controls (n = 6/group *P < 0.05; A). MAP in the Ephx2−/− DOCA-salt group was significantly lower than WT DOCA-salt. MAP measurements were also made in WT and Ephx2−/− DOCA-salt groups treated with the soluble epoxide hydrolase (sEH) inhibitor tAUCB (n = 6/group; B). MAP was significantly lower in WT and Ephx2−/− DOCA-salt + tAUCB groups compared with WT DOCA-salt, and there is no difference in MAP between Ephx2−/− and WT DOCA-salt tAUCB-treated groups. sEH protein expression was absent from the Ephx2−/− mice (C), and Cyp2c44 expression remained unchanged in both WT and Ephx2−/− kidney homogenates (D).

Fig. 2. Renal injury in DOCA-salt hypertension groups. Albuminuria increased significantly in WT DOCA-salt compared with WT control (n = 6/group *P < 0.05; A). Ephx2−/− DOCA-salt as well as WT DOCA-salt plus tAUCB mice displayed less albuminuria compared with WT DOCA-salt mice (+P < 0.05; A). B: Masson’s trichrome staining in kidney histological sections. C: Masson’s trichrome score (n = 6/group). Collagen deposition as assessed by the intensity of Masson’s trichrome stain was increased in WT DOCA-salt compared with control mice (*P < 0.05). Masson’s trichrome score was reduced in Ephx2−/− DOCA-salt compared with WT DOCA-salt (+P < 0.05).
Ephx2−/− control and Ephx2−/− DOCA-salt, but did increase in the WT DOCA-salt plus tAUCB group (P < 0.05 vs. WT DOCA-salt). Glomerular desmin has a reciprocal relationship with nephrin in that increased intensity is indicative of glomerular barrier injury (Fig. 4). Glomerular desmin intensity was increased in the WT DOCA-salt compared with WT control (P < 0.05). Fluorescent desmin intensity did not change between Ephx2−/− control and Ephx2−/− DOCA-salt mice.

Renal inflammation. Superarray analysis of inflammatory gene expression was examined and is shown in Fig. 5. DOCA-salt hypertension caused an increase in the expression of 25 proinflammatory genes in WT mice (P < 0.05 vs. WT control, Fig. 5 and supplemental Table 1; all supplementary material for this article can be found on the journal web site). DOCA-salt hypertension also caused a significant increase in the expression of 13 proinflammatory genes in Ephx2−/− mice (P < 0.05 vs. Ephx2−/− control). In addition, 24 inflammatory genes were significantly reduced in the Ephx2−/− DOCA-salt group compared with the WT DOCA-salt group (P < 0.05).

These data demonstrate that DOCA-salt hypertension increases renal inflammatory gene expression and that Ephx2 gene deficiency has an anti-inflammatory action.

Kidney sections were stained with ED-1 that recognizes CD68 to evaluate macrophage infiltration as an inflammatory indicator. The number of CD68-positive cells were counted and found to be increased significantly in the WT DOCA-salt compared with WT control group (346 ± 31, n = 6 vs. 136 ± 22 cell/mm², n = 5; P < 0.05). The increase in CD68-positive cells was significantly less in Ephx2−/− mice and averaged 131 ± 21 cell/mm² (n = 6) in Ephx2−/− control and 233 ± 54 cell/mm² (n = 5) in Ephx2−/− DOCA-salt mice. To further examine inflammation at the protein level, renal NF-κB activity was determined and is shown in Fig. 6A. Renal NF-κB activity increased significantly in the WT DOCA-salt group compared with WT control (*P < 0.05) whereas no change was noted between Ephx2−/− control and Ephx2−/− DOCA-salt groups. In addition, renal NF-κB activity was significantly reduced in the Ephx2−/− DOCA-salt compared with WT DOCA-salt group (+ P < 0.05). Another index of renal inflammation that was measured was urinary MCP-1 excretion (Fig. 6B). Control WT and Ephx2−/− mice displayed undetectable MCP-1 levels, and Ephx2−/− DOCA-salt mice excreted significantly lower levels of MCP-1 than WT DOCA-salt mice (+P < 0.05). Urinary MCP-1 excretion was also examined in the WT DOCA-salt plus tAUCB group and was significantly lower than the WT DOCA-salt mice (+P < 0.05). Taked together, these data demonstrate that inhibition the hydrolase domain of the sEH enzyme has renal anti-inflammatory actions in DOCA-salt hypertension.

Fig. 3. Glomerular nephrin immunofluorescence (A) and levels of relative fluorescent intensity (B) between DOCA-salt groups (n = 6/group). Glomerular nephrin fluorescent intensity decreased significantly in WT DOCA-salt (+P < 0.05 vs. WT control), while no change was seen in Ephx2−/− DOCA-salt mice (+P < 0.05 vs. Ephx2−/− control). Intensity in the WT DOCA-salt plus tAUCB increased significantly compared with WT DOCA-salt mice (+P < 0.05).
DISCUSSION

Chronic hypertension is a major risk factor for end-stage renal disease (1, 12). In addition, the Western diet includes a high salt intake, which in turn can exacerbate the disease condition (1, 5, 13). With the onset of hypertension, activation of the inflammatory pathway also occurs, which itself can possess adverse effects (15, 43, 54).

Interestingly, the major finding of the current study is that deletion of the Ephx2−/− gene provides renal protection by reducing renal injury and inflammation within the setting of salt-sensitive hypertension. Specifically, renal injury was attenuated as seen by reduced albuminuria, desmin, and increased nephrin levels. In addition, renal inflammation is reduced as seen by decreased macrophage infiltration, proinflammatory cytokine mRNA, as well as reduced renal NF-κB activity. These experimental findings are similar to previous findings in rat angiotensin salt-sensitive hypertension where urinary albumin excretion decreased and macrophage infiltration was reduced (20). The current study extends these findings to an angiotensin-independent animal model of salt-sensitive hypertension. Although blood pressure could be a factor contributing to the decrease in renal inflammation and injury, it is becoming clear in a number of renal and cardiovascular disease models that end-organ protection occurs independently of lowering blood pressure. In a recent study, sEH inhibition provided renal protection but did not lower blood pressure in diabetic rats that had salt-sensitive hypertension induced (40). Cisplatin-induced renal injury is also decreased by sEH inhibitor treatment independently of blood pressure changes (41). Experimental studies in Ephx2−/− mice have also demonstrated decreased inflammation and end-organ protection that is independent of blood pressure regulation (25, 35, 45). Since the

![Glomerular desmin expression in DOCA-salt hypertension groups.](image-url)

Fig. 4. Glomerular desmin expression in DOCA-salt hypertension groups. Glomerular desmin immunofluorescence (A) and levels of relative fluorescent intensity (B) between DOCA-salt groups (n = 6/group) are shown. Glomerular desmin fluorescent intensity increased significantly in WT DOCA-salt compared with WT control group (*P < 0.05), while no change was noted between Ephx2−/− DOCA-salt and control groups.
**Ephx2** gene is made up of a C-terminal hydrolase and N-terminal phosphatase domain, it remained unclear whether deletion of the phosphatase domain in **Ephx2** mice was contributing to the renal protection. By utilizing an inhibitor that is selective for the C-terminal hydrolase domain of sEH, the current study demonstrates that renal protection with salt-sensitive hypertension is due solely to inhibition of the hydrolase domain.

Maintenance of the filtration barrier is vital for proper renal structure and function. A reciprocal relationship exists between the podocyte proteins nephrin and desmin in that a decrease in nephrin and a rise in desmin correlate to renal damage (28, 53). This finding is further supported by a previous study from our laboratory that also examined these two proteins as indicators of renal damage (23). In addition, albuminuria increased significantly, which also supports that damage has occurred to the filtration barrier. Therefore, the current study demonstrates that deletion of the **Ephx2** gene ameliorates these indices of renal damage.

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There is growing support for the hypothesis that salt-sensitive hypertension is an inflammatory disease and therefore treatments that are anti-inflammatory can slow the progression of renal injury to end-stage renal disease (4, 49, 54). In the current study, we found that renal inflammation is enhanced both at the mRNA as well as protein level. The real-time PCR array clearly demonstrates that a significant inflammatory response occurs with DOCA-salt hypertension. In particular, ccr2, the receptor for MCP-1, increased significantly in the WT DOCA-salt group. Our urine analysis further corroborates this finding by showing increased urinary MCP-1 excretion with DOCA-salt hypertension in WT mice. Also, ICAM-1 and IL-1β mRNA were shown to be upregulated and have been previously found to be elevated with salt sensitive hypertension (31, 47, 54). Other inflammatory cytokines that increased with DOCA-salt hypertension include TNF-α as well as IL-6, and past studies have also shown these inflammatory markers to be elevated in other models of salt-sensitive hypertension (6–8, 31, 36). Interestingly, the real-time PCR array indicates that there is less inflammatory cytokine mRNA activated in the **Ephx2** DOCA-salt compared with WT DOCA-salt hypertension, which supports the notion that inhibition of epoxide breakdown by sEH provides renal protection.

To examine posttranslational activation of the inflammatory process, we measured renal NF-κB activity. Past studies have shown that EETs are anti-inflammatory and that EETs prevent the activation of NF-κB (9, 38). Acute inflammation induced by lipopolysaccharide injection is also decreased by sEH inhibition in mice (44). We have shown here that renal NF-κB activity...
activity is blunted in the Ephx2−/− DOCA-salt compared with WT DOCA-salt mice. We have also shown that renal macrophage infiltration is reduced in Ephx2−/− DOCA-salt compared with WT DOCA-salt mice. Hence, by preventing the degradation of epoxides by sEH, renal inflammation is blunted and may help restore renal structure with DOCA-salt hypertension.

The sEH enzyme is a homodimer, and each subunit is composed of two domains. The 35-kDa C-terminal domain imparts sEH activity, while the 25-kDa N-terminal domain has been classified as a phosphatase domain (37). Both domains have been well characterized, and evidence supports the notion that the C-terminal hydrolase domain plays a significant role in blood pressure regulation; however, a specific role of the N-terminal domain remains elusive (11, 17). To eliminate any involvement of the phosphatase domain in the blood pressure, anti-inflammatory, and renal-protective effects of Ephx2−/− gene deletion, a sEH inhibitor that selectively inhibits the C-terminal hydrolase domain was administered to WT and Ephx2−/− DOCA-salt mice. Our data demonstrate that it is the hydrolase domain that provides renal protection and that the phosphatase domain is not participating in this protection. Hence, silencing of the phosphatase domain in the Ephx2−/− mice does not contribute to the blood pressure-lowering effect and subsequent renal protection of sEH inhibition. Moreover, we observed no differences in MAP in Ephx2−/− DOCA-salt mice and WT DOCA-salt mice treated with the sEH inhibitor. Therefore, we have demonstrated that renal protection in the setting of salt-sensitive hypertension with total Ephx2 gene deletion is due to the C-terminal hydrolase domain.

The effects of Ephx2 gene deletion have also been examined in other vascular disease states, and these studies support the notion that sEH inhibitor effects are a consequence of inhibiting the activity of the C-terminal epoxide hydrolase domain (30, 32, 35, 45, 51). For instance, a recent study has identified Ephx2 as a heart failure susceptibility gene in the spontaneously hypertensive heart failure rat, which corresponds to human hypertension-associated heart failure (32). In addition, this study also tested the cardioprotective effects of Ephx2 gene deletion with angiotensin II administration as well as with pressure overload. Ephx2−/− mice, while similar to WT mice at baseline, were protected from cardiac arrhythmias following angiotensin II and demonstrated reduced ejection fraction decline with pressure overload (32). Ephx2−/− mice are also protected from end-organ damage that occurs with cerebral and cardiac ischemia and provided evidence that this protection was due to silencing the hydrolase domain of the sEH enzyme (25, 35, 45, 51). Taken as a whole, there is overwhelming evidence in experimental cardiovascular disease models that inhibition of the sEH enzyme hydrolase domain can provide significant protection from end-organ damage.

Polymorphisms of Ephx2 have been studied within the patient population and have been correlated to the incidences of such conditions as ischemic stroke, coronary artery disease, and vascular disease (14, 24, 26). In vitro studies by Koerner et al. (24) tested polymorphisms in the human Ephx2 gene and demonstrated that an alteration in sEH activity can affect neuronal survival postischemic injury. The Atherosclerosis Risk In Communities study identified a significant association between polymorphisms of Ephx2 and the incidence of coronary heart disease in Caucasian patients (26). These studies demonstrate that the Ephx2 gene has an important role in the development of cardiovascular disease, and therefore the potential therapeutic advantage of inhibiting this enzymatic pathway within cardiovascular disease states warrants further investigation.

Our study has demonstrated that renal injury and inflammation that occurs from salt-sensitive hypertension can be ameliorated by preventing the degradation of the epoxides and that this protection is due to the hydrolase domain of the Ephx2 gene. The protective effects of Ephx2 gene deletion include a reduction in blood pressure as well as an attenuation of glomerular injury which is correlated to a significant reduction in the inflammatory process. Therefore, the potential therapeutic benefit of using sEH inhibitors may have a two-fold protection, from blood pressure plus inflammation, that could alleviate the progression to end-stage renal disease associated with salt-sensitive hypertension.

GRANTS
This work was supported by National Institutes of Health Grants HL-59699, HL-074167, and DK-38226 and an American Heart Association Established Investigator Award to J. D. Imig.

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