Published in final edited form as:

Mol Cell Biochem. 2020 February ; 465(1-2): 37–51. doi:10.1007/s11010-019-03665-x.

***Ephx2*-gene deletion affects acetylcholine-induced relaxation in angiotensin-II infused mice: role of nitric oxide and CYP-epoxygenases**

Ahmad Hanif, PhD¹, Matthew L. Edin, PhD², Darryl C. Zeldin, MD², Mohammed A. Nayeem, PhD^{1,*}

¹Pharmaceutical Sciences, School of Pharmacy, Center for Basic and Translational Stroke Research. West Virginia University, Morgantown, WV

²Division of Intramural Research, NIEHS/NIH, Research Triangle Park, NC

Abstract

Previously, we showed that adenosine A_{2A} receptor-induces relaxation independent of NO in soluble epoxide hydrolase-null mice (*Am J Physiol Regul Integr Comp Physiol* 304: R23–R32, 2013). Currently, we hypothesize that *Ephx2*-gene deletion affects acetylcholine (Ach)-induced relaxation which is independent of A_{2A}AR but dependent on NO and CYP-epoxygenases. *Ephx2*^{-/-} aortas showed a lack of sEH (97.1%, *p*<0.05) but an increase in microsomal epoxide hydrolase (mEH, 37%, *p*<0.05) proteins compared to C57Bl/6 mice, and no change in CYP2C9 and CYP2J protein (*p*>0.05). Ach-induced response was tested with nitro-L-arginine methyl ester (L-NAME) NO-inhibitor; 10⁻⁴ M, *N*-(methylsulfonyl)-2-(2-propynyloxy)-benzenehexanamide (MS-PPOH) (CYP-epoxygenase inhibitor; 10⁻⁵ M), 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE, an epoxyeicosatrienoic acid-antagonist; 10⁻⁵ M), SCH-58261 (A_{2A}AR-antagonist; 10⁻⁶ M) and angiotensin-II (Ang-II, 10⁻⁶ M). In *Ephx2*^{-/-} mice, Ach-induced relaxation was not different from C57Bl/6 mice except at 10⁻⁵ M (92.75 ± 2.41 vs. 76.12 ± 3.34, *P*<0.05). However, Ach-induced relaxation was blocked by L-NAME (*Ephx2*^{-/-}: 23.74 ± 3.76% and C57Bl/6: 11.61 ± 2.82%), MS-PPOH (*Ephx2*^{-/-}: 48.16 ± 6.53% and C57Bl/6: 52.27 ± 7.47%) and 14,15-EEZE (*Ephx2*^{-/-}: 44.29 ± 8.33% and C57Bl/6: 39.27 ± 7.47%) vs. non-treated (*P*<0.05). But, it did not block by SCH-58261 (*Ephx2*^{-/-}: 68.75 ± 11.41% and C57Bl/6: 66.26 ± 9.43%, *P*>0.05) vs. non-treated (*P*>0.05). Interestingly, Ang-II attenuates less relaxation in *Ephx2*^{-/-} vs. C57Bl/6 mice (58.80 ± 7.81% vs. 45.92 ± 7.76, *P*<0.05). Our data suggests that Ach-induced relaxation in

*Corresponding author: Mohammed A. Nayeem, MSc, PhD., Associate Professor, Department of Pharmaceutical Sciences, School of Pharmacy, West Virginia University, Biomedical Research Building, 2nd floor, Room # 220, Health Science Center – North, 1 Medical Center Drive, PO Box 9530, Morgantown, WV 26506-9530, 304-293-4484-tel; 304-293-2576 - fax, mnayeem@hsc.wvu.edu.

AUTHOR CONTRIBUTION

Author contributions: M.A.N. conception, design of research, performing experiments, analysis drafting and editing; A.H. cooperating in the experimentations, reading, correction, editing and input; M.L.E. reading, correction, editing and input and D.C.Z. advising, reading, correction, editing, provide transgenic mice and input.

Publisher's Disclaimer: This Author Accepted Manuscript is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication but has not been copyedited or corrected. The official version of record that is published in the journal is kept up to date and so may therefore differ from this version.

DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

Ephx2^{-/-} mice depends on NO and CYP-epoxygenases but not on A_{2A} AR, and *Ephx2*-gene deletion attenuates less Ach-induced relaxation in Ang II-infused mice.

Keywords

Soluble epoxide hydrolase; Acetylcholine; Adenosine A_{2A} receptor; Nitric Oxide; CYP-epoxygenases; relaxation

Introduction

The nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) are released from vascular endothelium in human arteries [1, 2]. Epoxyeicosatrienoic acids (EETs) act as EDHFs, which are cytochrome-P450 epoxygenase-derived metabolites (from arachidonic acid, AA) [3, 4]. EETs can cause hyperpolarization in canine coronary arteries [5–7]. The soluble epoxide hydrolase (sEH), an enzyme which metabolize EETs to Dihydroxyeicosatrienoic acids (DHETs) and sEH inhibition or deletion could increase EET activity [8–13]. Cytochrome P-450-epoxygenases, including CYP2C (CYP2C29) and CYP2J (CYP2J2, CYP2J5) have been detected on endothelium [4, 11, 14–21]. Yang et al. [22] and Hanif et al. [11] showed up-regulation of CYP2J2 protects vascular endothelium against hypoxia-reoxygenation injury/ischemia reperfusion injury and enhanced coronary reactive hyperemic response [11, 22]. Endothelial CYP2C and CYP2J-epoxygenases are the main source of epoxide generation in the vascular system [4, 14, 23]. EETs present in small-resistance vessels, coronary arteries, and the aorta [11–13, 17, 24, 25]. In addition, EETs play an important role in the relaxation of isolated coronary arteries at a low concentration and are involved in increased coronary reactive hyperemic response [11–13, 24, 25]. EETs-induced responses have been seen in both bovine coronary arteries and mouse aortas which is inhibited by the 14,15-epoxyeicosa-5(*Z*)-enoic acid (EET antagonist) [18, 19, 25, 26].

The sEH enzyme (*Ephx2* gene) is commonly present in various mammalian tissues, including the kidney, intestine, liver, heart, coronary arteries, and aortas [9–13, 25, 27–29]. sEH metabolizes four forms of EET regioisomers to their corresponding diols [27, 30]. In the presence of CYP-epoxygenases, conversion of arachidonic acids into epoxides then into diols by sEH, diminishes the beneficial effects on cardiovascular functions. Inhibition or deletion of sEH enables beneficial epoxides to accumulate and stay for longer periods after they are generated from arachidonic acids [12, 13, 24, 25]. Several reports indicate that sEH inhibition could be a promising approach for controlling of hypertension and vascular diseases [31–34]. However, Hercule, et al., indicated that there is a possibility CYP450-eicosanoids activate eNOS and release NO in sEH^{-/-} and WT mouse mesenteric arteries [35, 36]. NO and EETs maintain a stable arterial wall viscosity during blood flow increase and this endothelial adaptive regulation is lost during essential hypertension [37]. The sEH inhibitor improved endothelium-dependent relaxations to acetylcholine, assessed by myography in isolated coronary arteries. This improvement was related to a restoration of EETs and NO pathways, as shown by the increased inhibitory effects of the NOS and cytochrome P-450 epoxygenase inhibitors l-NA and MSPPOH on these relaxations [38]. In contrast, we demonstrated that adenosine A_{2A} receptor-induced relaxation response was

NO-independent in sEH^{-/-} mice, where CYP450-epoxygenases derived EETs may play an important role in mouse aortic relaxation [25]. In addition, sEH inhibition reduced the blood pressure in Ang-II-induced hypertension [34, 39] and sEH deletion restricted the reduction of coronary reactive hyperemic response in Ang-II-infused sEH^{-/-} vs. WT mice (unpublished data from our lab). Taking all of these findings into consideration, we hypothesized that *Ephx2*-gene deletion affects Ach-induced relaxation which is independent of A_{2A}AR but dependent on NO and CYP-epoxygenases.

Materials and Methods

Animals

Sinal et al. [40 2071] described the generation of *Ephx2*^{-/-} mice. *Ephx2*^{-/-} and C57Bl/6 mice were provided by Dr. Darryl Zeldin, NIEHS/NIH). West Virginia University Institutional Animal Care and Use Committee approved all animal care and experimentation protocols, and were in accordance with the principles and guidelines of the NIH's *Guide for the Care and Use of Laboratory Animals*. We used both male and female mice (14–18 wk. old) equally in our study.

Materials

Phenylephrine and ACh (Sigma Chemicals, St. Louis, MO) and Angiotensin II (Human), BACHEM (Bubendorf, Switzerland) dissolved in distilled water. 14,15-EEZE and MS-PPOH (Cayman Chemicals), SCH 58261 and L-NAME (Sigma Chemicals, St. Louis, MO) were dissolved in DMSO [25]. CYP2J2 and Cyp2c29 (Dr. Zeldin, NIEHS/NIH), sEH (Santa Cruz Biotechnology) and mEH (Institut für Pharmakologie und Toxikologie, Zürich, Switzerland) and β-actin (Santa Cruz Biotechnology) antibodies were obtained and used for Western blot experiments [25].

Protein extraction, gel electrophoresis, and Western blot analysis

Mice were euthanized with pentobarbital sodium (100 mg/kg ip). According to our previously described protocol [17–20, 41–48], after thoracotomy, the aortas were gently removed and cleaned of fat and connective tissue [25]. In brief, aortas from both *Ephx2*^{-/-} and C57Bl/6 mice were treated with 1 ml of lysis buffer for protein extraction [25]. Gel electrophoresis and Western blot analysis were done according to the protocol described by us protocol [17–20, 41–48]. Following blocking with nonfat dry milk, the nitrocellulose membranes were incubated with polyclonal primary antibodies raised against for CYP2J2 and CYP2C29 (Dr. Zeldin, NIEHS/NIH), monoclonal sEH (Santa Cruz Biotechnology) microsomal epoxide hydrolase (mEH, Institut für Pharmakologie und Toxikologie, Zürich, Switzerland) and β-actin (Santa Cruz Biotechnology) [25]. We used the secondary antibody, horseradish peroxidase-conjugated anti-rabbit IgG [25]. The membranes were developed using enhanced chemiluminescence (Amersham Biosciences) and exposed to X-ray film for appropriate time [25]. Mouse does not have CYP2J2, but antibody likely to recognize some of the mouse CYP2Js.

Isometric Tension Muscle Bath Experiments

Ephx2^{-/-} and C57Bl/6 mice were euthanized with pentobarbital sodium (100 mg/kg intraperitoneally) [25]. The aorta was removed after thoracotomy and cut into rings of 3–4 mm in length [25]. The rings were suspended between the two wire hooks [25]. Two rings were suspended in organ baths containing 10 ml of modified Krebs-Henseleit buffer [17–21, 25, 42, 44, 49]. After the equilibration period (60 min), tissues were contracted with KCl (50 mM) to assess the viability of the tissue [25]. Rings were then constricted with phenylephrine (PE; 10⁻⁷ M), and tension was monitored continuously with a fixed range precision force transducer (125C; BIOPAC Systems) connected to amplifiers (Data Acquisition system 100B; BIOPAC Systems) [25]. Data was recorded using MP100 WSW, BIOPAC digital acquisition system and analyzed using Acknowledge 3.5.7 software (BIOPAC Systems) [25]. Ach-dose (10⁻¹² – 10⁻⁵ M) dependent response experiments were conducted as previously described by our laboratory with adenosine agonist (NECA), adenosine A₁ receptor agonist (CCPA) and adenosine A_{2A} receptor agonist (CGS 21680) [17–21, 25, 42, 44, 49]. The aortic rings were washed several times with Krebs-Henseleit solution and allowed to equilibrate for 30 min before the experimental protocol began. Results are expressed as percentages downward or upward on PE-induced precontraction [25].

Effect of nitric oxide inhibitor (L-NAME) on ACh-induced response in *Ephx2*^{-/-} and C57Bl/6 mice. Concentration-response curves (CRCs) were obtained by cumulative addition of drugs in 1-log increments as described by us [17–21, 25, 42, 44, 49]. A single CRC was constructed for each ring in parallel in pairs of rings from either *Ephx2*^{-/-} and C57Bl/6 mice in the same organ bath [25]. L-NAME (10 μM & 100 μM) was added 30 min before the PE contraction and was present throughout the ACh CRC.

Effects of N-(methylsulfonyl)-2-(2-propynyloxy)-benzenhexanamide (MS-PPOH) (CYP-epoxygenase inhibitor on Ach-induced CRC in *Ephx2*^{-/-} and C57Bl/6 mice. Ach-CRC was obtained with and without MS-PPOH (10 μM), and MS-PPOH was added 30 min before the PE contraction and was present throughout the ACh CRC [25]. Similar as described earlier by us [11, 12, 17, 18, 21, 44, 49].

Effects of epoxyeicosatrienoic acids (EETs) receptor antagonist 14,15-EEZE on Ach-induced CRC in *Ephx2*^{-/-} and C57Bl/6 mice. Ach-CRC was obtained with and without 14,15-EEZE (10 μM), which was added 30 min before the PE contraction and was present throughout the ACh CRC [25]. Similar as described earlier by us [17–19, 25].

Effect of A_{2A} AR antagonists (SCH 58261) on Ach-induced CRC in *Ephx2*^{-/-} and C57Bl/6 mice. Ach-induced CRC obtained as described above [25]. SCH 58261 (1 μM) was added 30 min before PE contraction and was present throughout the experiment as described earlier by us [17, 18, 25].

Effects of Angiotensin-II (Ang-II, 1 μM) on Ach-induced CRC in *Ephx2*^{-/-} and C57Bl/6 mice. Ach-CRC was obtained with and without Ang-II (1 μM), and Ang-II was added 30 min before the PE contraction and was present throughout the ACh CRC [25]. Similar as described earlier by us [20, 50].

Statistical analysis

The data reported as means $\pm$ SE. One-way ANOVA was used to compare difference among groups, and two-way ANOVA was used for repeated measures, followed by Tukey post hoc test to compare the Ach-induced vascular responses of non-treated vs. inhibitor/antagonist (L-NAME, MS-PPOH, 14,15-EEZE, SCH 58261 and Ang-II)-treated *Ephx2*^{-/-} and C57Bl/6 mice [25]. Differences (*Ephx2*^{-/-} vs. C57Bl/6), non-treated vs. (L-NAME, MS-PPOH, 14,15-EEZE, SCH 58261 and Ang-II)-treated *Ephx2*^{-/-} and C57Bl/6 mice, and L-NAME-treated vs. (MS-PPOH, 14,15-EEZE, SCH 58261 and Ang-II)-treated *Ephx2*^{-/-} and C57Bl/6 mice were considered significant when $P < 0.05$ [25]. Further, densitometry of Western blot analysis (mEH, CYP2J2, Cyp2c29 and sEH) expressed as means $\pm$ SE in arbitrary units [25]. All the statistical analyses performed using GraphPad Prism statistical package [25].

Results

Expression of sEH, mEH, CYP2J2 and Cyp2c29 proteins in aortas from *Ephx2*^{-/-} and C57Bl/6 mice

Western blot analysis showed 97% less sEH (~60 kDa) protein in *Ephx2*^{-/-} vs. C57Bl/6 mice ($P < 0.05$, Fig. 1A) but showed 37% more mEH (~50 kDa) protein in *Ephx2*^{-/-} vs. C57Bl/6 mice ($P < 0.05$, Fig. 1B). There was no significant difference observed between *Ephx2*^{-/-} vs. C57Bl/6 mice ($P > 0.05$, Fig. 2A & 2B) concerning CYP2C29 (~60 kDa) and CYP2J (~60 kDa) proteins.

Concentration Response Curve (CRC) for ACh and the effect of nitric oxide inhibitor in *Ephx2*^{-/-} and C57Bl/6 mice

ACh caused a concentration (10^{-7} – 10^{-5} M)-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice, but the response was not significantly different (ACh- 10^{-7} & 10^{-6} M, $P > 0.05$) except (ACh- 10^{-5} M, $P < 0.05$) between *Ephx2*^{-/-} and C57Bl/6 mice (92.75 ± 2.41 vs. 76.12 ± 3.34 , Fig. 3). In addition, L-NAME (10 μ M & 100 μ M) had altered vascular response significantly ($P < 0.05$) in both C57Bl/6 (10 μ M L-NAME at ACh 10^{-5} M, 36.25 ± 2.64 vs non-treated 76.12 ± 3.34 , $P < 0.05$, Fig. 4A) and *Ephx2*^{-/-} (10 μ M L-NAME at ACh 10^{-5} M, 48.79 ± 8.54 vs non-treated 92.75 ± 2.41 , $P < 0.05$, Fig. 4B). Whereas, with 100 μ M L-NAME treated C57Bl/6 (at ACh 10^{-5} M, 11.61 ± 2.82 vs non-treated 76.12 ± 3.34 , $P < 0.05$, Fig. 4A) and *Ephx2*^{-/-} (100 μ M L-NAME at ACh 10^{-5} M, 23.74 ± 3.76 vs non-treated 92.75 ± 2.41 , $P < 0.05$, Fig. 4B). There is a significant difference in dose (10^{-7} to 10^{-5} M ACh)-dependent response observed between (100 μ M L-NAME)-treated vs. non-treated *Ephx2*^{-/-} and C57Bl/6 mice $P < 0.05$, Fig. 5). In addition, there is a significant difference in dose (10^{-7} to 10^{-5} M ACh)-dependent response observed between (100 μ M L-NAME)-treated *Ephx2*^{-/-} vs. (100 μ M L-NAME)-treated C57Bl/6 mice $P < 0.05$, Fig. 5). At ACh 10^{-5} M, there is a significant difference between 100 μ M L-NAME treated C57Bl/6 (11.61 ± 2.82) vs. 100 μ M L-NAME treated *Ephx2*^{-/-} (23.74 ± 3.76 , $P < 0.05$, Fig. 5).

CRC for Ach with or without MS-PPOH (CYP-epoxygenases inhibitor, 10^{-5} M) treated C57Bl/6 and *Ephx2*^{-/-} mice

At 10^{-5} M Ach, MS-PPOH reduced the Ach-induced dose-dependent relaxation in C57Bl/6 (52.27 ± 7.47 vs. non-treated 76.12 ± 3.34 , $P < 0.05$, Fig. 6A) as well as in *Ephx2*^{-/-} mice (48.16 ± 6.53 vs. non-treated 92.75 ± 2.41 , $P < 0.05$, Fig. 6B). No significant change was observed between MS-PPOH-treated C57Bl/6 vs. MS-PPOH-treated *Ephx2*^{-/-} mice ($P > 0.05$, Fig. 7). However, there was a significant difference in the reduction of Ach-induced relaxation observed between MS-PPOH (10^{-5} M)-treated vs. L-NAME (10^{-4} M)-treated C57Bl/6 mice (at 10^{-5} M Ach, MS-PPOH: 52.27 ± 7.47 vs. L-NAME: 11.61 ± 2.82 , $P < 0.05$, Fig. 7). Similarly, there was a significant difference in the reduction of Ach-induced relaxation observed between MS-PPOH (10^{-5} M)-treated vs. L-NAME (10^{-4} M)-treated *Ephx2*^{-/-} mice (at 10^{-5} M Ach, MS-PPOH: 48.16 ± 6.53 vs. L-NAME: 23.74 ± 3.76 , $P < 0.05$, Fig. 7). In addition, there was a significant difference in the reduction of Ach-induced dose (10^{-7} to 10^{-5} M Ach)-dependent relaxation observed between MS-PPOH-treated vs. L-NAME-treated C57Bl/6 and *Ephx2*^{-/-} mice ($P < 0.05$, Fig. 7).

CRC for Ach with or without 14,15-EEZE (EETs receptor antagonist, 10^{-5} M) treated C57Bl/6 and *Ephx2*^{-/-} mice

At 10^{-5} M Ach, 14,15-EEZE reduced the Ach-induced dose-dependent relaxation in C57Bl/6 (39.27 ± 7.47 vs. non-treated 76.12 ± 3.34 , $P < 0.05$, Fig. 8A) as well as in *Ephx2*^{-/-} mice (44.29 ± 8.33 vs. non-treated 92.75 ± 2.41 , $P < 0.05$, Fig. 8B). There was no significant change observed between 14,15-EEZE-treated C57Bl/6 vs. 14,15-EEZE-treated *Ephx2*^{-/-} mice ($P > 0.05$, Fig. 9). However, there was a significant difference in the reduction of Ach-induced relaxation between 14,15-EEZE-treated vs. L-NAME-treated C57Bl/6 mice (at 10^{-5} M Ach, 14,15-EEZE: 39.27 ± 7.47 vs. L-NAME: 11.61 ± 2.82 , $P < 0.05$, Fig. 9). Similarly, there was a significant difference in the reduction of Ach-induced relaxation observed between 14,15-EEZE (10^{-5} M)-treated vs. L-NAME (10^{-4} M)-treated *Ephx2*^{-/-} mice (at 10^{-5} M Ach, 14,15-EEZE: 44.29 ± 8.33 vs. L-NAME: 23.74 ± 3.76 , $P < 0.05$, Fig. 9). In addition, there was a significant difference in the reduction of Ach-induced dose (10^{-7} to 10^{-5} M Ach)-dependent relaxation observed between 14,15-EEZE-treated vs. L-NAME-treated C57Bl/6 and *Ephx2*^{-/-} mice ($P < 0.05$, Fig. 9).

CRC for Ach with and without SCH 58261 (A_{2A} AR-antagonist) in *Ephx2*^{-/-} and C57Bl/6 mice

Ach produced a concentration dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. A_{2A} AR antagonist, SCH 58261 produced no significant change in Ach-induced dose-dependent relaxation ($P > 0.05$, Figs. 10A & 10B). However, there was a significant difference in the reduction of Ach-induced relaxation observed between SCH 58261 (10^{-6} M)-treated vs. L-NAME (10^{-4} M)-treated C57Bl/6 mice (at 10^{-5} M Ach, SCH 58261: 66.26 ± 9.43 vs. L-NAME: 11.61 ± 2.82 , $P < 0.05$, Fig. 11). Similarly, there was a significant difference in the reduction of Ach-induced relaxation observed between SCH-58261-treated vs. L-NAME-treated *Ephx2*^{-/-} mice (at 10^{-5} M Ach, SCH-58261: 68.75 ± 11.41 vs. L-NAME: 23.74 ± 3.76 , $P < 0.05$, Fig. 11). In addition, there was a significant difference was

observed between SCH 58261-treated vs. L-NAME-treated C57Bl/6 and *Ephx2*^{-/-} mice ($P < 0.05$, Fig. 11).

CRC for Ach and the effects of Ang-II (10⁻⁶ M) in *Ephx2*^{-/-} and C57Bl/6 mice.

Ach produced a concentration-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Ang-II (1 μ M) attenuates Ach-induced dose-dependent relaxation in both C57Bl/6 (at Ach 10⁻⁷ - 10⁻⁵ M; 9.06 $\pm$ 5.35, 32.71 $\pm$ 9.26, 45.92 $\pm$ 7.76 vs. non-treated 37.24 $\pm$ 4.21, 59.29 $\pm$ 4.19, 76.12 $\pm$ 3.34, $P < 0.05$, Fig. 12A) and *Ephx2*^{-/-} (at Ach 10⁻⁷ - 10⁻⁵ M; 21.34 $\pm$ 4.23, 50.92 $\pm$ 5.95, 58.80 $\pm$ 4.81 vs. non-treated 51.70 $\pm$ 3.91, 65.12 $\pm$ 9.95, 92.75 $\pm$ 2.41, $P < 0.05$, Fig. 12B) mice. Interestingly, Ang-II treatment attenuates more Ach-induced dose-dependent relaxation in C57Bl/6 (at Ach 10⁻⁷ - 10⁻⁵ M; 9.06 $\pm$ 5.35, 32.71 $\pm$ 9.26, 45.92 $\pm$ 7.76, Fig. 13 A) vs. *Ephx2*^{-/-} mice (at Ach 10⁻⁷ - 10⁻⁵ M; 21.34 $\pm$ 4.23, 50.92 $\pm$ 5.95, 58.80 $\pm$ 4.81 $P < 0.05$, Fig. 13 A). Nevertheless, there was a significant difference in the reduction of Ach-induced relaxation observed between Ang-II (10⁻⁶ M)-treated vs. L-NAME (10⁻⁴ M)-treated C57Bl/6 mice (at 10⁻⁵ M Ach, Ang-II: 45.92 $\pm$ 7.76 vs. L-NAME: 11.61 $\pm$ 2.82, $P < 0.05$, Fig. 13 B). Similarly, there was a significant difference in the reduction of Ach-induced relaxation observed between Ang-II (10⁻⁶ M)-treated vs. L-NAME (10⁻⁴ M)-treated *Ephx2*^{-/-} mice (at 10⁻⁵ M Ach, Ang-II: 58.80 $\pm$ 4.81 vs. L-NAME: 23.74 $\pm$ 3.76, $P < 0.05$, Fig. 13 B). In addition, there was a significant difference in the reduction of Ach-induced dose (10⁻⁷ to 10⁻⁵ M Ach)-dependent relaxation observed between Ang-II treated vs. L-NAME-treated C57Bl/6 and *Ephx2*^{-/-} mice ($P < 0.05$, Fig. 13 B).

Discussion

The *Ephx2*^{-/-} mice show no sEH protein expression compared to C57Bl/6 mice, whereas, mEH (microsomal epoxide hydrolase, Ephx1) was significantly upregulated in *Ephx2*^{-/-} compared to C57Bl/6 mice (Figs 1A & B). mEH is often expressed at lower levels than sEH and displays much lower rates of EET hydrolysis *in vitro*; however, it is anchored to ER membranes, interacts with P450 epoxygenases, and may participate in a coupled reaction to hydrolyze EETs during low, basal rates of EET formation [51–53]. Importantly, in sEH-deficient mice or tissues, mEH functionally compensates to hydrolyze the majority of EETs produced [53, 54]. The effect of mEH on EET is biologically relevant, as mEH/sEH-null hearts have significantly improved post-ischemic heart function compared to sEH null hearts [53]. Thus, the compensatory upregulation of mEH observed in our *Ephx2*^{-/-} mice may significantly affect EETs hydrolysis in the current study. Therefore, mEH is upregulated in *Ephx2*^{-/-} compared to C57Bl/6 mice, and there is no change in CYP2C29 and CYP2J protein expression in *Ephx2*^{-/-} compared to C57Bl/6 mice (Figs 2A & B). This is the first study to investigate the Ach-induced dose-dependent vascular (aortic) response interaction between CYP-epoxygenases, soluble epoxide hydrolase, and nitric oxide in *Ephx2*^{-/-} and C57Bl/6 mice. We are reporting some novel findings due to this interaction. First, L-NAME attenuates most of the Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Thus, it is clear that Ach-induced relaxation is almost all dependent on NO. However, attenuation of Ach-induced dose-dependent relaxation due to L-NAME in *Ephx2*^{-/-} is less than C57Bl/6 mice. Therefore, sEH nullification in *Ephx2*^{-/-} mice plays

role in resisting the reduction of Ach-induced dose-dependent relaxation due to L-NAME in *Ephx2*^{-/-} compared to C57Bl/6 mice. (2) CYP-epoxygenase inhibitor (MS-PPOH) was able to partially attenuate Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice, thus, demonstrating that CYP-epoxygenases are partially involved in Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. (3) The EETs antagonist (14,15-EEZE) was also able to partially attenuate Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Therefore, EETs seem to be involved in Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. (4) The A_{2A} AR-antagonist (SCH 58261) did not alter Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Hence, it appears that Ach-induced dose-dependent relaxation is independent of A_{2A} AR but dependent on NO. (5) Ang-II also attenuates less Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice. According to our data, sEH plays an important role in the Ach-induced dose-dependent regulation of vascular response.

Genes related to Cytochrome P450-epoxygenases polymorphism have been identified in various communities regarding its role in cardiovascular function and in blood pressure regulation [55–62]. Also, individuals with variations in EPHX2 and ω-hydroxylase genes have an increased-risk of coronary heart disease, ischemic stroke, restenosis, diabetes heart, heart failure, ischemic stroke in Caucasians, Chinese, and in the black Americans with increase in blood pressure [25] [55, 62–66]. Seubert et al., reported that differential renal sEH gene expression in pre-hypertensive, hypertensive, and spontaneously hypertensive rats (SHR) [25] [67], and mice lacking or inhibiting sEH have enhanced coronary reactive hyperemic response in *Ephx2*^{-/-} and *t*-AUCB-treated mice [12, 13, 25]. However, Hercule, et al., indicated that CYP450-eicosanoids activate endothelial NO synthase (eNOS) and NO release in *Ephx2*^{-/-} and WT mouse mesenteric arteries [35, 36]. In contrast, we demonstrated that adenosine A_{2A} receptor-induced relaxation was NO independent in sEH-null mice, where CYP450-epoxygenases-derived EETs play an important role in aortic relaxation [25]. In addition, sEH inhibition reduced the blood pressure in Ang-II-induced hypertension [34, 39] and sEH deletion restricted the reduction of coronary reactive hyperemic response in Ang-II-infused sEH^{-/-} vs. WT mice (un-published data from our lab). Therefore, we hypothesized that *Ephx2* gene deletion attenuates Ach-induced relaxation which is independent of A_{2A}AR but dependent on NO and CYP-epoxygenases.

Role of sEH in Ach-induced relaxation. Ach-induced dose-dependent aortic relaxation in *Ephx2*^{-/-} was not different ($P < 0.05$) from C57Bl/6 mice except at 10⁻⁵ M Ach ($P < 0.05$, Fig. 3). This is very different from adenosine (5'-*N*-ethylcarboxamidoadenosine, adenosine agonist, NECA) and adenosine A_{2A} receptor (A_{2A} AR agonist, CGS 21680)-induced dose-dependent aortic relaxation in sEH^{-/-} compared to C57Bl/6 ($P < 0.05$) [25], and this A_{2A} AR-induced vascular response was independent of NO and COX [17, 18, 21, 25]. ACh data between *Ephx2*^{-/-} and C57Bl/6 mice suggest that there is no relationship between ACh and the presence or absence of sEH except at 10⁻⁵ M of Ach, which is similar to as we reported earlier from our lab [25].

Role of NO in Ach-induced relaxation. L-NAME, a nitric oxide synthase inhibitor (100 μM) had almost completely blocked ACh-induced vascular response in both *Ephx2*^{-/-} and

C57Bl/6 mice ($P < 0.05$, Fig. 4A & B). However, there is a slightly significant difference observed at 10^{-4} M of L-NAME in Ach-induced dose-dependent vascular response between *Ephx2*^{-/-} and C57Bl/6 mice ($P < 0.05$, Fig. 5). This indicates that the deletion of the *Ephx2* gene confers resistance to the reduction of Ach-induced dose-dependent aortic relaxation caused by L-NAME treatment in *Ephx2*^{-/-} compared to C57Bl/6 mice. Due to deletion of *Ephx2* gene, sEH enzyme activity is aborted and the conversion of EETs into dihydroxyeicosatrienoic acids (DHETs) may be stopped, causing epoxides to accumulate and stay for longer periods after they are generated from arachidonic acids [13, 25]. This is a compelling approach for controlling hypertension and vascular diseases [12, 13, 24, 25, 31–34]. Also, EETs have been demonstrated to increase the release of NO in cultured bovine aortic endothelial cells (BEACs), and EETs/DHETs can induce endothelial NO release to modulate vascular tone [36, 68]. Interestingly our observation shows deletion of *Ephx2* gene results in a resistance to the reduction of Ach-induced dose-dependent aortic relaxation causing by L-NAME treatment in *Ephx2*^{-/-} compared to C57Bl/6 mice, and may be due to a higher availability of EETs in *Ephx2*^{-/-} compared to C57Bl/6 mice. Similarly, we have seen in NECA (adenosine) and CGS 21680 (A_{2A}AR agonist)-induced dose-dependent aortic relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice [25], as well as enhanced coronary reactive hyperemic response in *Ephx2*^{-/-} vs. C57Bl/6 mice [13]. In addition to that, NECA and CGS 21680-significantly enhanced aortic relaxation in eNOS^{-/-} vs. C57Bl/6 mice and this relaxation was dependent on A_{2A} AR through CYP-epoxygenases-EETs pathway but not eNOS [21]. Therefore, according to the current study, CYP-epoxygenases-EETs pathway is both A_{2A} AR-dependent but NO-independent and NO-dependent but A_{2A} AR-independent.

Role of CYP-epoxygenases in Ach-induced relaxation. MS-PPOH (CYP-epoxygenases inhibitor) was able to partially block Ach-induced dose-dependent aortic relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice compared to non-treated mice (Figs 6A & B). No difference was found between *Ephx2*^{-/-} and C57Bl/6 mice. MS-PPOH has significantly less blocking action than L-NAME-treated *Ephx2*^{-/-} and C57Bl/6 mice (Fig. 7). These findings indicate that CYP-epoxygenases are also involved in Ach-induced dose-dependent aortic relaxation. Similarly, as reported in mouse mesenteric arteries [36]. In contrast, we have observed NECA (adenosine) and CGS 21680 (A_{2A}AR agonist)-induced dose-dependent aortic relaxation independent of NO and COX in sEH^{-/-} compared to C57Bl/6 mice [25] as well as enhanced coronary reactive hyperemic response in *Ephx2*^{-/-} vs. C57Bl/6 mice [13]. However, CYP-epoxygenases are involved in Ach-induced dose-dependent aortic relaxation, similarly as reported in mouse mesenteric arteries [36].

Role of EETs in Ach-induced relaxation. 14,15-EEZE (EETs antagonist) was able to block partially Ach-induced dose-dependent aortic relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice compared to non-treated mice (Figs 8A & B). There was no difference between 14,15-EEZE-treated *Ephx2*^{-/-} and 14,15-EEZE-treated C57Bl/6 mice. 14,15-EEZE has significantly less reduction in blocking action than L-NAME treated *Ephx2*^{-/-} and C57Bl/6 mice (Fig. 9). That means EETs are involved in Ach-induced dose-dependent aortic relaxation. Similarly as reported in mouse mesenteric arteries [36].

Role of adenosine A_{2A} receptor in Ach-induced relaxation. SCH-58261 (1 μ M, A_{2A}AR antagonist) was not able to block Ach-induced dose-dependent aortic relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice (Figs. 10A & B). In addition, there is a significant difference between SCH-58261-treated *Ephx2*^{-/-} and SCH-58261-treated C57Bl/6 mouse Ach-induced dose-dependent aortic vascular responses (Fig. 11). These data are in agreement with our earlier reports that NECA (adenosine) and CGS 21680 (A_{2A}AR agonist)-induced dose-dependent aortic relaxation are independent of NO and COX in sEH^{-/-} and C57Bl/6 mice [17, 18, 21, 25], as well as enhanced coronary reactive hyperemic response in sEH^{-/-} vs. C57Bl/6 mice [13]. In addition, the difference between the blocking action of L-NAME vs. non-treated *Ephx2*^{-/-}/C57Bl/6 mice and SCH-58261-treated *Ephx2*^{-/-}/C57Bl/6 mice are the same (Fig. 11).

Effect of Ang-II in Ach-induced relaxation. Ang-II (1 μ M) was able to partially block Ach-induced dose-dependent aortic relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice compared to non-treated mice (Figs. 12A & B), and there is a significant difference was found between the blocking action of Ang-II in *Ephx2*^{-/-} compared to C57Bl/6 mice (Fig. 13A). This shows that the absence of sEH activity due to lack of *Ephx2* gene in *Ephx2*^{-/-} compared to C57Bl/6 mice has an advantage in reducing the blocking action of Ang-II in Ach-induced dose-dependent aortic relaxation. As mentioned earlier, the inhibition or deletion of sEH makes beneficial epoxides accumulate and reside for longer periods after they are generated from arachidonic acids, and sEH inhibition may be a compelling approach for controlling hypertension and vascular diseases [12, 13, 24, 25, 31–34]. Also, Ang II up-regulated sEH [69], and sEH inhibition blocked Ang II-induced increase in blood pressure in rats [70]. Further, Ang II blocking action on Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice has significantly lesser reduction in blocking action than L-NAME treated *Ephx2*^{-/-} and C57Bl/6 mice (Fig. 13 B). Both Ang-II and L-NAME blocked Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} and C57Bl/6 mice... However, in the absence of sEH in *Ephx2*^{-/-} mice, Ang-II and L-NAME blocking action is significantly less than the blocking action in C57Bl/6 mice. Therefore, we conclude that inhibition or deletion of sEH in mice may reduce the toxic action of both Ang-II and L-NAME in Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice.

Conclusion

In conclusion, inhibition or absence of sEH through deletion of the *Ephx2* gene appears to be critical for acetylcholine-induced dose-dependent relaxation while challenged by Angiotensin-II or L-NAME. When the sEH enzyme was absent in *Ephx2*^{-/-} mice, Ach-induced dose-dependent relaxation was similar to C57Bl/6 mice except at 10⁻⁵ M of Ach. However, when the Ach-induced dose-dependent relaxation was challenged by Ang-II or L-NAME, absence of sEH made a significant difference in the resistance to block in Ach-induced dose-dependent relaxation compared to the presence of sEH in C57Bl/6 mice. Therefore, we conclude that inhibition or deletion of sEH in mice may reduce the toxic action of both Ang-II and L-NAME in Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice.

Summary

Deletion of sEH in *Ephx2*^{-/-} results in mEH (Ephx1) upregulation. Therefore the absence of sEH in the *Ephx2*^{-/-} mice results in mEH having a small role in hydrolyzing EETs into DHETs in the current study as mentioned by others [51, 52, 54]. L-NAME attenuates most of the Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Thus, it is clear that Ach-induced relaxation is almost solely dependent on NO. However, attenuation of Ach-induced dose-dependent relaxation due to L-NAME in *Ephx2*^{-/-} is less than C57Bl/6 mice. CYP-epoxygenase inhibitor (MS-PPOH) was able to partially attenuates Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Thus, it demonstrates that CYP-epoxygenases are partially involved in Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. EETs antagonist (14,15-EEZE) was also able to partially attenuate Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. Therefore, EETs seem to be involved in Ach-induced dose-dependent relaxation in both *Ephx2*^{-/-} and C57Bl/6 mice. However, A_{2A} AR-antagonist (SCH 58261) did not alter Ach-induced dose-dependent relaxation in either *Ephx2*^{-/-} and C57Bl/6 mice. Hence, it appears that Ach-induced dose-dependent relaxation is independent of A_{2A} AR but dependent on NO. Ang-II also attenuates less Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice. According to our data, sEH plays an important role in the in Ach-induced dose-dependent regulation of vascular response. Therefore, we conclude that inhibition or deletion of sEH in mice may reduce the toxic action of both Ang-II and L-NAME in Ach-induced dose-dependent relaxation in *Ephx2*^{-/-} compared to C57Bl/6 mice.

ACKNOWLEDGMENTS

This work supported by National Institutes of Health Grant HL-114559 to M. A. Nayeem and National Institute of Environmental Health Sciences Grant Z01 ES025034 to D. C. Zeldin. In addition, we are very much thankful to Ms. Brandy J. Wilmoth, B.S., RVT (Biology Technician) for performing tissue bath experiments and thankful to Amanda G. Ammer, PhD. (Research Assistant Professor), Department of Microbiology, Immunology and Cell Biology for editing and correcting this manuscript.

References

1. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K and Takeshita A (1997) Importance of endothelium-derived hyperpolarizing factor in human arteries. *J Clin Invest* 100:2793–9. doi: 10.1172/JCI119826 [PubMed: 9389744]
2. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM and Weston AH (2002) EDHF: bringing the concepts together. *Trends Pharmacol Sci* 23:374–80. [PubMed: 12377579]
3. Campbell WB, Gebremedhin D, Pratt PF and Harder DR (1996) Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res* 78:415–23. [PubMed: 8593700]
4. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I and Busse R (1999) Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 401:493–7. [PubMed: 10519554]
5. Capdevila JH, Falck JR and Harris RC (2000) Cytochrome P450 and arachidonic acid bioactivation. Molecular and functional properties of the arachidonate monooxygenase. *J Lipid Res* 41:163–81. [PubMed: 10681399]
6. Oltman CL, Weintraub NL, VanRollins M and Dellsperger KC (1998) Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ Res* 83:932–9. [PubMed: 9797342]

7. Sarkis A, Lopez B and Roman RJ (2004) Role of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids in hypertension. *Curr Opin Nephrol Hypertens* 13:205–14. [PubMed: 15202615]
8. Spector AA, Fang X, Snyder GD and Weintraub NL (2004) Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog Lipid Res* 43:55–90. [PubMed: 14636671]
9. Hanif A, Edin ML, Zeldin DC, Morisseau C, Falck JR, Ledent C, Tilley SL and Nayeem MA (2017) Reduced coronary reactive hyperemia in mice was reversed by the soluble epoxide hydrolase inhibitor (t-AUCB): Role of adenosine A2A receptor and plasma oxylipins. *Prostaglandins Other Lipid Mediat* 131:83–95. doi: S1098-8823(17)30085-0 [pii] 10.1016/j.prostaglandins.2017.09.001 [PubMed: 28890385]
10. Hanif A, Edin ML, Zeldin DC, Morisseau C, Falck JR and Nayeem MA (2017) Vascular Endothelial Over-Expression of Human Soluble Epoxide Hydrolase (Tie2-seH Tr) Attenuates Coronary Reactive Hyperemia in Mice: Role of Oxylipins and omega-Hydroxylases. *PLoS One* 12:e0169584. doi: 10.1371/journal.pone.0169584 PONE-D-16-40923 [pii] [PubMed: 28056085]
11. Hanif A, Edin ML, Zeldin DC, Morisseau C, Falck JR and Nayeem MA (2017) Vascular endothelial overexpression of human CYP2J2 (Tie2-CYP2J2 Tr) modulates cardiac oxylipin profiles and enhances coronary reactive hyperemia in mice. *PLoS One* 12:e0174137. doi: 10.1371/journal.pone.0174137 PONE-D-16-50098 [pii] [PubMed: 28328948]
12. Hanif A, Edin ML, Zeldin DC, Morisseau C and Nayeem MA (2016) Effect of Soluble Epoxide Hydrolase on the Modulation of Coronary Reactive Hyperemia: Role of Oxylipins and PPARgamma. *PLoS One* 11:e0162147. doi: 10.1371/journal.pone.0162147 PONE-D-16-28211 [pii] [PubMed: 27583776]
13. Hanif A, Edin ML, Zeldin DC, Morisseau C and Nayeem MA (2016) Deletion of soluble epoxide hydrolase enhances coronary reactive hyperemia in isolated mouse heart: role of oxylipins and PPARgamma. *Am J Physiol Regul Integr Comp Physiol* 311:R676–R688. doi: 10.1152/ajpregu.00237.2016 ajpregu.00237.2016 [pii] [PubMed: 27488890]
14. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC and Liao JK (1999) Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285:1276–9. [PubMed: 10455056]
15. Zeldin DC and Liao JK (2000) Reply: cytochrome P450-derived eicosanoids and the vascular wall. *Trends Pharmacol Sci* 21:127–128. doi: S0165-6147(00)01454-1 [pii] [PubMed: 10740284]
16. Zeldin DC, Wohlford-Lenane C, Chulada P, Bradbury JA, Scarborough PE, Roggli V, Langenbach R and Schwartz DA (2001) Airway inflammation and responsiveness in prostaglandin H synthase-deficient mice exposed to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 25:457–65. [PubMed: 11694451]
17. Nayeem MA, Poloyac SM, Falck JR, Zeldin DC, Ledent C, Ponnoth DS, Ansari HR and Mustafa SJ (2008) Role of CYP epoxygenases in A2A AR-mediated relaxation using A2A AR-null and wild-type mice. *Am J Physiol Heart Circ Physiol* 295:H2068–78. [PubMed: 18805895]
18. Nayeem MA, Ponnoth DS, Boegehold MA, Zeldin DC, Falck JR and Mustafa SJ (2009) High-salt diet enhances mouse aortic relaxation through adenosine A2A receptor via CYP epoxygenases. *Am J Physiol Regul Integr Comp Physiol* 296:R567–74. [PubMed: 19109366]
19. Pradhan I, Zeldin DC, Ledent C, Mustafa JS, Falck JR and Nayeem MA (2014) High salt diet exacerbates vascular contraction in the absence of adenosine A(2)A receptor. *J Cardiovasc Pharmacol* 63:385–94. doi: 10.1097/FJC.000000000000058 [PubMed: 24390173]
20. Ponnoth DS, Nayeem MA, Kunduri SS, Tilley SL, Zeldin DC, Ledent C and Mustafa SJ (2012) Role of omega-hydroxylase in adenosine-mediated aortic response through MAP kinase using A2A-receptor knockout mice. *Am J Physiol Regul Integr Comp Physiol* 302:R400–8. doi: ajpregu.00481.2011 [pii] 10.1152/ajpregu.00481.2011 [PubMed: 22160543]
21. Nayeem MA, Zeldin DC, Boegehold MA and Falck JR (2011) Salt modulates vascular response through adenosine A(2A) receptor in eNOS-null mice: role of CYP450 epoxygenase and soluble epoxide hydrolase. *Mol Cell Biochem* 350:101–11. doi: 10.1007/s11010-010-0686-0 [PubMed: 21161333]
22. Yang B, Graham L, Dikalov S, Mason RP, Falck JR, Liao JK and Zeldin DC (2001) Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. *Mol Pharmacol* 60:310–20. [PubMed: 11455018]

23. Rosolowsky M and Campbell WB (1996) Synthesis of hydroxyeicosatetraenoic (HETEs) and epoxyeicosatrienoic acids (EETs) by cultured bovine coronary artery endothelial cells. *Biochim Biophys Acta* 1299:267–77. [PubMed: 8555273]
24. Fang X, Kaduce TL, Weintraub NL, Harmon S, Teesch LM, Morisseau C, Thompson DA, Hammock BD and Spector AA (2001) Pathways of epoxyeicosatrienoic acid metabolism in endothelial cells. Implications for the vascular effects of soluble epoxide hydrolase inhibition. *J Biol Chem* 276:14867–74. [PubMed: 11278979]
25. Nayeem MA, Pradhan I, Mustafa SJ, Morisseau C, Falck JR and Zeldin DC (2013) Adenosine A2A receptor modulates vascular response in soluble epoxide hydrolase-null mice through CYP-epoxygenases and PPARgamma. *Am J Physiol Regul Integr Comp Physiol* 304:R23–32. doi: ajpregu.00213.2012 [pii] 10.1152/ajpregu.00213.2012 [PubMed: 23152114]
26. Gauthier KM, Deeter C, Krishna UM, Reddy YK, Bondlela M, Falck JR and Campbell WB (2002) 14,15-Epoxyeicosa-5(Z)-enoic acid: a selective epoxyeicosatrienoic acid antagonist that inhibits endothelium-dependent hyperpolarization and relaxation in coronary arteries. *Circ Res* 90:1028–36. [PubMed: 12016270]
27. Newman JW, Morisseau C and Hammock BD (2005) Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog Lipid Res* 44:1–51. [PubMed: 15748653]
28. Wang P, Meijer J and Guengerich FP (1982) Purification of human liver cytosolic epoxide hydrolase and comparison to the microsomal enzyme. *Biochemistry* 21:5769–76. [PubMed: 6185139]
29. Yu Z, Davis BB, Morisseau C, Hammock BD, Olson JL, Kroetz DL and Weiss RH (2004) Vascular localization of soluble epoxide hydrolase in the human kidney. *Am J Physiol Renal Physiol* 286:F720–6. [PubMed: 14665429]
30. Morisseau C and Hammock BD (2005) Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. *Annu Rev Pharmacol Toxicol* 45:311–33. [PubMed: 15822179]
31. Elmarakby AA, Faulkner J, Al-Shabrawey M, Wang MH, Maddipati KR and Imig JD (2011) Deletion of soluble epoxide hydrolase gene improves renal endothelial function and reduces renal inflammation and injury in streptozotocin-induced diabetes. *Am J Physiol Regul Integr Comp Physiol*. doi: ajpregu.00759.2010 [pii]10.1152/ajpregu.00759.2010
32. Elmarakby AA, Ibrahim AS, Faulkner J, Mozaffari MS, Liou GI and Abdelsayed R (2011) Tyrosine kinase inhibitor, genistein, reduces renal inflammation and injury in streptozotocin-induced diabetic mice. *Vascul Pharmacol*. doi: S1537-1891(11)00074-7 [pii] 10.1016/j.vph.2011.07.007
33. Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP and Hammock BD (2005) Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc Natl Acad Sci U S A* 102:9772–7. [PubMed: 15994227]
34. Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC and Kroetz DL (2000) Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res* 87:992–8. [PubMed: 11090543]
35. Gao J, Bellien J, Gomez E, Henry JP, Dautreaux B, Bounoure F, Skiba M, Thuillez C and Richard V (2011) Soluble epoxide hydrolase inhibition prevents coronary endothelial dysfunction in mice with renovascular hypertension. *J Hypertens* 29:1128–35. doi: 10.1097/HJH.0b013e328345ef7b [PubMed: 21451419]
36. Hercule HC, Schunck WH, Gross V, Seringer J, Leung FP, Weldon SM, da Costa Goncalves A, Huang Y, Luft FC and Gollasch M (2009) Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice. *Arterioscler Thromb Vasc Biol* 29:54–60. doi: 10.1161/ATVBAHA.108.171298 [pii] [PubMed: 18927469]
37. Roca F, Bellien J, Jacob M and Joannides R (2019) Endothelium-dependent adaptation of arterial wall viscosity during blood flow increase is impaired in essential hypertension. *Atherosclerosis* 285:102–107. doi: S0021-9150(19)30364-8 [pii] 10.1016/j.atherosclerosis.2019.04.208 [PubMed: 31051413]
38. Roche C, Besnier M, Cassel R, Harouki N, Coquerel D, Guerrot D, Nicol L, Loizon E, Remy-Jouet I, Morisseau C, Mulder P, Ouvrard-Pascaud A, Madec AM, Richard V and Bellien J (2015) Soluble epoxide hydrolase inhibition improves coronary endothelial function and prevents the development of cardiac alterations in obese insulin-resistant mice. *Am J Physiol Heart Circ*

- Physiol 308:H1020–9. doi: 10.1152/ajpheart.00465.2014 ajpheart.00465.2014 [pii] [PubMed: 25724490]
39. Jung O, Brandes RP, Kim IH, Schweda F, Schmidt R, Hammock BD, Busse R and Fleming I (2005) Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension* 45:759–65. [PubMed: 15699457]
 40. Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR and Gonzalez FJ (2000) Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* 275:40504–10. [PubMed: 11001943]
 41. Yadav VR, Hong KL, Zeldin DC and Nayeem MA (2016) Vascular endothelial over-expression of soluble epoxide hydrolase (Tie2-sEH) enhances adenosine A1 receptor-dependent contraction in mouse mesenteric arteries: role of ATP-sensitive K⁺ channels. *Mol Cell Biochem* 422:197–206. doi: 10.1007/s11010-016-2821-z 10.1007/s11010-016-2821-z [pii] [PubMed: 27629787]
 42. Pradhan I, Ledent C, Mustafa SJ, Morisseau C and Nayeem MA (2015) High salt diet modulates vascular response in A2AAR (+/+) and A2AAR (–/–) mice: role of sEH, PPARγ, and K⁺ ATP channels. *Mol Cell Biochem* 404:87–96. doi: 10.1007/s11010-015-2368-4 [PubMed: 25739357]
 43. Kunduri SS, Mustafa SJ, Ponnoth DS, Dick GM and Nayeem MA (2013) Adenosine A1 receptors link to smooth muscle contraction via CYP4a, protein kinase C-α, and ERK1/2. *J Cardiovasc Pharmacol* 62:78–83. doi: 10.1097/FJC.0b013e3182919591 [PubMed: 23519140]
 44. Ponnoth DS, Nayeem MA, Tilley SL, Ledent C and Jamal Mustafa S (2012) CYP-epoxygenases contribute to A2A receptor-mediated aortic relaxation via sarcolemmal KATP channels. *Am J Physiol Regul Integr Comp Physiol* 303:R1003–10. doi: ajpregu.00335.2012 [pii] 10.1152/ajpregu.00335.2012 [PubMed: 23019210]
 45. Nayeem MA (2004) Sublethal simulated ischemia promotes delayed resistance against ischemia via ATP-sensitive (K⁺) channels in murine myocytes: role of PKC and iNOS. *Antioxid Redox Signal* 6:375–83. [PubMed: 15025939]
 46. Nayeem MA and Mustafa SJ (2002) Protein kinase C isoforms and A1 adenosine receptors in porcine coronary smooth muscle cells. *Vascul Pharmacol* 39:47–54. [PubMed: 12616990]
 47. Nayeem MA, Elliott GT, Shah MR, Hastillo-Hess SL and Kukreja RC (1997) Monophosphoryl lipid A protects adult rat cardiac myocytes with induction of the 72-kD heat shock protein: a cellular model of pharmacologic preconditioning. *J Mol Cell Cardiol* 29:2305–10. [PubMed: 9281461]
 48. Nayeem MA, Hess ML, Qian YZ, Loesser KE and Kukreja RC (1997) Delayed preconditioning of cultured adult rat cardiac myocytes: role of 70- and 90-kDa heat stress proteins. *Am J Physiol* 273:H861–8. [PubMed: 9277504]
 49. Nayeem MA, Zeldin DC, Boegehold MA, Morisseau C, Marowsky A, Ponnoth DS, Roush KP and Falck JR (2010) Modulation by salt intake of the vascular response mediated through adenosine A(2A) receptor: role of CYP epoxygenase and soluble epoxide hydrolase. *Am J Physiol Regul Integr Comp Physiol* 299:R325–33. doi: ajpregu.00823.2009 [pii] 10.1152/ajpregu.00823.2009 [PubMed: 20427718]
 50. Yadav VR, Nayeem MA, Tilley SL and Mustafa SJ (2015) Angiotensin II stimulation alters vasomotor response to adenosine in mouse mesenteric artery: role for A1 and A2B adenosine receptors. *Br J Pharmacol* 172:4959–69. doi: 10.1111/bph.13265 [PubMed: 26227882]
 51. Morisseau C and Hammock BD (2013) Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu Rev Pharmacol Toxicol* 53:37–58. doi: 10.1146/annurev-pharmtox-011112-140244 [PubMed: 23020295]
 52. Decker M, Adamska M, Cronin A, Di Giallono F, Burgener J, Marowsky A, Falck JR, Morisseau C, Hammock BD, Gruzdev A, Zeldin DC and Arand M (2012) EH3 (ABHD9): the first member of a new epoxide hydrolase family with high activity for fatty acid epoxides. *J Lipid Res* 53:2038–45. doi: 10.1194/jlr.M024448 jlr.M024448 [pii] [PubMed: 22798687]
 53. Edin ML, Hamedani BG, Gruzdev A, Graves JP, Lih FB, Arbes SJ 3rd, Singh R, Orjuela Leon AC, Bradbury JA, DeGraff LM, Hoopes SL, Arand M and Zeldin DC (2018) Epoxide hydrolase 1 (EPHX1) hydrolyzes epoxyeicosanoids and impairs cardiac recovery after ischemia. *J Biol Chem* 293:3281–3292. doi: 10.1074/jbc.RA117.000298 RA117.000298 [pii] [PubMed: 29298899]

54. Marowsky A, Burgener J, Falck JR, Fritschy JM and Arand M (2009) Distribution of soluble and microsomal epoxide hydrolase in the mouse brain and its contribution to cerebral epoxyeicosatrienoic acid metabolism. *Neuroscience* 163:646–61. doi: 10.1016/j.neuroscience.2009.06.033 S0306-4522(09)01058-6 [pii] [PubMed: 19540314]
55. Fava C, Montagnana M, Almgren P, Hedblad B, Engstrom G, Berglund G, Minuz P and Melander O (2010) The common functional polymorphism –50G>T of the CYP2J2 gene is not associated with ischemic coronary and cerebrovascular events in an urban-based sample of Swedes. *J Hypertens* 28:294–9. doi: 10.1097/HJH.0b013e328333097e [PubMed: 19851119]
56. Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L and DiPetrillo K (2008) Validation of volume-pressure recording tail-cuff blood pressure measurements. *Am J Hypertens* 21:1288–91. doi: ajh2008301 [pii]10.1038/ajh.2008.301 [PubMed: 18846043]
57. Jie Z, Hong K, Jianhong T, Biao C, Yongmei Z and Jingchuan L (2010) Haplotype analysis of the CYP2J2 gene associated with myocardial infarction in a Chinese Han population. *Cell Biochem Funct* 28:435–9. doi: 10.1002/cbf.1661 [PubMed: 20597138]
58. Lee CR, North KE, Bray MS, Couper DJ, Heiss G and Zeldin DC (2007) CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study. *Pharmacogenet Genomics* 17:349–58. doi: 10.1097/FPC.0b013e32809913ea 01213011-200705000-00006 [pii] [PubMed: 17429317]
59. Theken KN and Lee CR (2007) Genetic variation in the cytochrome P450 epoxigenase pathway and cardiovascular disease risk. *Pharmacogenomics* 8:1369–83. [PubMed: 17979511]
60. Wang CP, Hung WC, Yu TH, Chiu CA, Lu LF, Chung FM, Hung CH, Shin SJ, Chen HJ and Lee YJ (2010) Genetic variation in the G-50T polymorphism of the cytochrome P450 epoxigenase CYP2J2 gene and the risk of younger onset type 2 diabetes among Chinese population: potential interaction with body mass index and family history. *Exp Clin Endocrinol Diabetes* 118:346–52. doi: 10.1055/s-0029-1243604 [PubMed: 20140850]
61. Xu Y, Ding H, Peng J, Cui G, Liu L, Cianflone K and Wang DW (2011) Association between polymorphisms of CYP2J2 and EPHX2 genes and risk of coronary artery disease. *Pharmacogenet Genomics* 21:489–94. doi: 10.1097/FPC.0b013e3283485eb2 [PubMed: 21642892]
62. Zordoky BN and El-Kadi AO (2010) Effect of cytochrome P450 polymorphism on arachidonic acid metabolism and their impact on cardiovascular diseases. *Pharmacol Ther* 125:446–63. doi: S0163-7258(10)00002-1 [pii] 10.1016/j.pharmthera.2009.12.002 [PubMed: 20093140]
63. Burdon KP, Lehtinen AB, Langefeld CD, Carr JJ, Rich SS, Freedman BI, Herrington D and Bowden DW (2008) Genetic analysis of the soluble epoxide hydrolase gene, EPHX2, in subclinical cardiovascular disease in the Diabetes Heart Study. *Diab Vasc Dis Res* 5:128–34. doi: 3047 [pii]10.3132/dvdr.2008.021 [PubMed: 18537101]
64. Kullmann S, Binner P, Rackebrandt K, Hüge A, Haltern G, Lankisch M, Futh R, von Hodenberg E, Bestehorn HP and Scheffold T (2009) Variation in the human soluble epoxide hydrolase gene and risk of restenosis after percutaneous coronary intervention. *BMC Cardiovasc Disord* 9:48. doi: 1471-2261-9-48 [pii]10.1186/1471-2261-9-48 [PubMed: 19814804]
65. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, Couper DJ, Heiss G and Zeldin DC (2006) Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Hum Mol Genet* 15:1640–9. [PubMed: 16595607]
66. Monti J, Fischer J, Paskas S, Heinig M, Schulz H, Gosele C, Heuser A, Fischer R, Schmidt C, Schirdewan A, Gross V, Hummel O, Maatz H, Patone G, Saar K, Vingron M, Weldon SM, Lindpaintner K, Hammock BD, Rohde K, Dietz R, Cook SA, Schunck WH, Luft FC and Hubner N (2008) Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. *Nat Genet* 40:529–37. doi: ng.129 [pii]10.1038/ng.129 [PubMed: 18443590]
67. Seubert JM, Xu F, Graves JP, Collins JB, Sieber SO, Paules RS, Kroetz DL and Zeldin DC (2005) Differential renal gene expression in prehypertensive and hypertensive spontaneously hypertensive rats. *Am J Physiol Renal Physiol* 289:F552–61. [PubMed: 15798089]
68. Wang H, Lin L, Jiang J, Wang Y, Lu ZY, Bradbury JA, Lih FB, Wang DW and Zeldin DC (2003) Upregulation of endothelial nitric-oxide synthase by endothelium-derived hyperpolarizing factor involves mitogen-activated protein kinase and protein kinase C signaling pathways. *J Pharmacol Exp Ther* 307:753–64. [PubMed: 12975498]

69. Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C, Hammock BD, Shyy JY and Zhu Y (2007) Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. *Proc Natl Acad Sci U S A* 104:9018–23. doi: 0703229104 [pii]10.1073/pnas.0703229104 [PubMed: 17495027]
70. Honetschlagerova Z, Sporkova A, Kopkan L, Huskova Z, Hwang SH, Hammock BD, Imig JD, Kramer HJ, Kujal P, Vernerova Z, Chabova VC, Tesar V and Cervenka L (2011) Inhibition of soluble epoxide hydrolase improves the impaired pressure-natriuresis relationship and attenuates the development of hypertension and hypertension-associated end-organ damage in Cyp1a1-Ren-2 transgenic rats. *J Hypertens* 29:1590–601. doi: 10.1097/HJH.0b013e328349062f [PubMed: 21720266]

Fig. 1. Representative Western blots and densitometric analysis for sEH (~60 kDa; A) and mEH (~50 kDa; B) proteins in aortas of *Ephx2*^{-/-} and C57Bl/6 mice. Values are expressed as means ± SE. **P* < 0.05, C57Bl/6 compared with *Ephx2*^{-/-} mouse aortas, *n* = 8 per group.

Fig. 2. Representative Western blots and densitometric analysis for Cyp2c29 (~60 kDa; A) and CYP2J2s (~60 kDa; B) proteins in aortas of *Ephx2*^{-/-} and C57Bl/6 mice. Values expressed as means ± SE. $P > 0.05$, C57Bl/6 compared with *Ephx2*^{-/-} mouse aortas, $n = 8$ per group.

Fig. 3.

Acetylcholine (ACh)-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. * $P < 0.05$ between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10^{-5} M of ACh. Otherwise, there is no significant difference found in (ACh)-induced dose-dependent vascular response between C57Bl/6 and *Ephx2*^{-/-} mice ($P > 0.05$). Values expressed as means $\pm$ SE, $n = 8$ per group.

Fig. 4.

Effect of L-NAME (10^{-5} M & 10^{-4} M; A) on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 mice. * $P < 0.05$ between non-treated C57Bl/6 and 10^{-5} M L-NAME treated C57Bl/6 mice. + $P < 0.05$ between non-treated C57Bl/6 and 10^{-4} M L-NAME treated C57Bl/6 mice. Similarly, Effect of L-NAME (10^{-5} M & 10^{-4} M; B) on Ach-induced dose-dependent vascular response in aortic rings of *Ephx2*^{-/-} mice. * $P < 0.05$ between non-treated *Ephx2*^{-/-} and 10^{-5} M L-NAME treated *Ephx2*^{-/-} mice. + $P < 0.05$ between non-treated *Ephx2*^{-/-} and 10^{-4} M L-NAME treated *Ephx2*^{-/-} mice. Values expressed as means $\pm$ SE, $n = 8$ per group.

Fig. 5.

Effect of *Ephx2* deletion on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. \$*P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of Ach. Also, effect of L-NAME (10⁻⁴ M) on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between non-treated C57Bl/6 and 10⁻⁴ M L-NAME treated C57Bl/6 mice. @*P* < 0.05 between non-treated *Ephx2*^{-/-} and 10⁻⁴ M L-NAME treated *Ephx2*^{-/-} mice. Also, the effect of L-NAME (10⁻⁴ M) on Ach-induced dose-dependent vascular response between C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between non-treated *Ephx2*^{-/-} and 10⁻⁵ M L-NAME treated *Ephx2*^{-/-} mice. *@*P* < 0.05 between the 10⁻⁴ M L-NAME treated *C57Bl/6 and 10⁻⁴ M L-NAME treated @*Ephx2*^{-/-} mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 6.

Effect of MS-PPOH (10^{-5} M; A & B) on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and Ephx2^{-/-} mice. * $P < 0.05$ between non-treated C57Bl/6 and 10^{-5} M MS-PPOH treated C57Bl/6 mice. * $P < 0.05$ between non-treated Ephx2^{-/-} and 10^{-5} M MS-PPOH treated Ephx2^{-/-} mice [25]. Values expressed as means $\pm$ SE, $n = 8$ per group.

Fig. 7.

Effect of *Ephx2* deletion on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of Ach. Also, effect of L-NAME and MS-PPOH on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. #*P* < 0.05 between non-treated C57Bl/6 and L-NAME treated C57Bl/6 mice. @*P* < 0.05 between non-treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. \$*P* < 0.05 between non-treated C57Bl/6 and MS-PPOH treated C57Bl/6 mice. %*P* < 0.05 between non-treated *Ephx2*^{-/-} and MS-PPOH treated *Ephx2*^{-/-} mice. \$#*P* < 0.05 between MS-PPOH treated C57Bl/6 and L-NAME treated C57Bl/6 mice. %@*P* < 0.05 between MS-PPOH treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 8. Effect of 14,15-EEZE (10⁻⁵ M; A & B) on ACh-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between non-treated C57Bl/6 and 10⁻⁵ M 14,15-EEZE treated C57Bl/6 mice. **P* < 0.05 between non-treated *Ephx2*^{-/-} and 10⁻⁵ M 14,15-EEZE treated *Ephx2*^{-/-} mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 9.

Effect of *Ephx2* deletion on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of Ach. Also, effect of L-NAME and 14,15-EEZE on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. #*P* < 0.05 between non-treated C57Bl/6 and L-NAME treated C57Bl/6 mice. @*P* < 0.05 between non-treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. \$*P* < 0.05 between non-treated C57Bl/6 and 14,15-EEZE treated C57Bl/6 mice. %*P* < 0.05 between non-treated *Ephx2*^{-/-} and 14,15-EEZE treated *Ephx2*^{-/-} mice. \$#*P* < 0.05 between 14,15-EEZE treated C57Bl/6 and L-NAME treated C57Bl/6 mice. %@*P* < 0.05 between 14,15-EEZE treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 10.

Effect of SCH 58261 (10^{-6} M; A & B) on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. * $P < 0.05$ between non-treated C57Bl/6 and 10^{-6} M SCH 58261 treated C57Bl/6 mice. * $P < 0.05$ between non-treated *Ephx2*^{-/-} and 10^{-6} M SCH 58261 treated *Ephx2*^{-/-} mice. Values expressed as means $\pm$ SE, $n = 8$ per group.

Fig. 11.

Effect of *Ephx2* deletion on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of Ach. Also, effect of L-NAME and SCH 58261 on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. #*P* < 0.05 between non-treated or SCH 58261 treated C57Bl/6 and L-NAME treated C57Bl/6 mice. @*P* < 0.05 between non-treated or SCH 58261 treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 12.

Effect of Ang-II (10^{-6} M; A & B) on ACh-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. * $P < 0.05$ between non-treated C57Bl/6 and 10^{-6} M Ang-II treated C57Bl/6 mice. * $P < 0.05$ between non-treated *Ephx2*^{-/-} and 10^{-6} M Ang-II treated *Ephx2*^{-/-} mice. Values expressed as means $\pm$ SE, $n = 8$ per group.

Fig. 13 A.

Effect of *Ephx2* deletion on ACh-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. \$*P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of ACh. Also, effect of Ang-II (10⁻⁶ M) on ACh-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between non-treated C57Bl/6 and Ang-II treated C57Bl/6 mice. +*P* < 0.05 between non-treated *Ephx2*^{-/-} and Ang-II treated *Ephx2*^{-/-} mice. Also, the effect of Ang-II (10⁻⁶ M) on ACh-induced dose-dependent vascular response between C57Bl/6 and *Ephx2*^{-/-} mice. ***P* < 0.05 between the 10⁻⁶ M Ang-II treated *C57Bl/6 and 10⁻⁶ M Ang-II treated *Ephx2*^{-/-} mice, *n* = 8 per group.

Fig. 13 B.

Effect of *Ephx2* deletion on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. **P* < 0.05 between C57Bl/6 vs. *Ephx2*^{-/-} mice only at 10⁻⁵ M of Ach. Also, effect of L-NAME and Ang-II on Ach-induced dose-dependent vascular response in aortic rings of C57Bl/6 and *Ephx2*^{-/-} mice. #*P* < 0.05 between non-treated or Ang-II treated C57Bl/6 and L-NAME treated C57Bl/6 mice. @*P* < 0.05 between non-treated or Ang-II treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. \$@*P* < 0.05 between Ang-II treated *Ephx2*^{-/-} and L-NAME treated *Ephx2*^{-/-} mice. %#*P* < 0.05 between Ang-II treated C57Bl/6 and L-NAME treated C57Bl/6 mice. Values expressed as means ± SE, *n* = 8 per group.

Fig. 14.

Flow diagram: Comparison between *Ephx2*^{-/-} vs. C57Bl/6 mice treated with Acetylcholine, Acetylcholine + L-NAME, Acetylcholine + MS-PPOH/14,15-EEZE, Acetylcholine + SCH-58261 and Acetylcholine + Ang-II.