Adenosine A2A receptor modulates vascular response in soluble epoxide hydrolase-null mice through CYP-epoxygenases and PPARγ

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The soluble epoxide hydrolase (sEH) enzyme is detected in a variety of mammalian tissues, including the liver, kidney,
intestine, and blood vessels (40, 56, 60). Within these tissues, sEH metabolizes epoxide-containing compounds to their corresponding diols (31, 40). Conversion of arachidonic acid epoxides to diols by sEH diminishes the beneficial cardiovascular properties of these epoxyeicosanoids. Inhibition of sEH causes EETs to accumulate and be retained for longer periods after they are formed (9). Some reports provide further evidence that sEH inhibition may be an effective approach for the treatment of hypertension and diseases associated with vascular inflammation (7, 8, 18, 47, 61). Also, the targeted disruption of the sEH gene in male mice lowers systolic blood pressure (51). Recently, we found that there is a possible link between the upregulation of A2A AR, CYP-epoxygenases, and down-regulation of sEH with adenosine-induced relaxation in mouse aorta (38). Also, we found that there is a possible link between the down-regulation of A2A AR, CYP-epoxygenases, and up-regulation of sEH with 5'-N-ethylcarboxamidoadenosine (NECA)-induced contraction in mouse aorta (38, 39). Therefore, we hypothesized that the lack of sEH enhances adenosine-induced vascular relaxation through A2A AR via CYP-epoxygenases and PPARγ.

MATERIALS AND METHODS

The generation of sEH−/− mice was described by Sinal et al. (51). sEH−/− and sEH+/+ mice were provided by Dr. Zeldin, National Institute of Environmental Health Sciences/National Institutes of Health (NIH). All animal care and experimentation protocols were approved and carried out in accordance with the West Virginia University Institutional Animal Care and Use Committee and were in accordance with the principles and guidelines of the NIH’s Guide for the Care and Use of Laboratory Animals. Both male and female mice (14–18 wk old) in equal ratio were used in our study.

Protein extraction, gel electrophoresis, and Western blot analysis. Mice were killed with pentobarbital sodium (100 mg/kg ip). According to our previously described protocol (36–39), after thoracotomy, the aorta was gently removed and cleaned of fat and connective tissue. In brief, aortas from both sEH−/− and sEH+/+ mice were treated with 1 ml of lysis buffer for protein extraction. Gel electrophoresis and Western blot analysis were done according to the protocol described by us (36–39). Following blocking with nonfat dry milk, the nitrocellulose membranes were incubated with polyclonal primary antibodies for CYP2J5 (Dr. Zeldin, NEIHS/NIH), A1 AR (Sigma Chemicals), A2A AR (Alpha Diagnostic), CYP4A, PPARα, PPARγ, and β-actin (Santa Cruz Biotechnology). The secondary antibody, horse-radish peroxidase-conjugated anti-rabbit IgG, was used. The membranes were developed using enhanced chemiluminescence (Amer sham Biosciences) and exposed to X-ray film for appropriate time.

sEH−/− and sEH+/+ aortic rings. After thoracotomy the aorta was gently removed, cleaned of fat and connective tissue, and cut transversely into rings of 3–4 mm in length. Care was taken not to damage the endothelium. The rings were hung vertically between two wire hooks. Two rings were suspended in organ baths containing 10 ml of modified Krebs-Henseleit buffer (36–39). After the equilibration period (60 min), tissues were contracted with KCl (50 mM) to assess the viability of the tissue. 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). Data were recorded using MP100 WSW, BIOPAC digital acquisition system and analyzed using Acknowledge 3.5.7 software (BIOPAC Systems). The functionality of endothelium was tested with ACh (10−7 M) on precontracted (PE) rings, as previously described by our laboratory (36–39). 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 % downward or upward on PE-induced precontraction.

Effect of nitric oxide inhibitor (t-NAME) on ACh-induced response in sEH−/− and sEH+/+ mice. Concentration-response curves (CRCs) were obtained by cumulative addition of drugs in 1-log increments as described by us (36–39). A single CRC was constructed for each ring in parallel in pairs of rings from either sEH−/− or sEH+/+ in the same organ bath. t-NAME (100 μM) was added 30 min before the PE contraction and was present throughout the ACh CRC.

Effect of A2A AR antagonists (ZM 241385 or SCH 58261) on NECA-induced CRC in sEH−/− and sEH+/+ mice. Adenosine analog NECA-induced CRC was obtained as described above. ZM 241385 or SCH 58261 (1 μM) was added 30 min before PE contraction and was present throughout the experiment as described above.

Effects of nitric oxide (t-NAME)/epoxyeicosatrienoic acids (EETs) receptor antagonist 14,15-EEZE on CGS 21680-induced CRC in sEH−/− and sEH+/+ mice. CGS 21680 (A2A AR agonist)-CRC was obtained with and without t-NAME (100 μM) or 14,15-epoxyeicosatrienoic acid (14,15-EEZE; 10 μM), as described above.

Effects of sEH inhibitors on CGS 21680-induced CRC in sEH−/− and sEH+/+ mice. CGS 21680 CRC was obtained with and without 12-(3-adamantan-1-yl-ureido) dodecanoic acid (AUDA, a sEH inhibitor; 10 μM) or trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyl]-benzoic acid (t-AUCB, a sEH inhibitor; 10 μM) as described above.

CRC for CGS 21680 and PPARα-agonist (GW 7647) and the effect of PPARα-agonist (T0070907) on sEH−/− and sEH+/+ mice. CGS 21680-induced CRC was compared with GW 7647-induced CRC in sEH−/− and sEH+/+ mice. Also, CGS 21680-induced CRC was obtained with and without T0070907 (PPARα-agonist; 0.1 μM), as described above.

Chemicals, drugs, and antibodies. Phenylephrine and ACh (Sigma Chemicals, St. Louis, MO) were dissolved in distilled water. NECA and CGS 21680 (Sigma Chemicals, St. Louis, MO) were dissolved in 100% DMSO as 10 mM stock solutions, which were followed by serial dilutions in distilled water. T0070907, GW 7647 (Cayman Chemicals), 14,15-EEZE (Dr. Falck), and AUDA and t-AUCB (Dr. Montreuil) were dissolved in DMSO. CYP2J5 (Dr. Zeldin, NEIHS/NIH), A1 AR (Sigma Chemicals), CYP4A, PPARα, and PPARγ (Santa Cruz Biotechnology), and A2A AR (Alpha Diagnostic) antibodies were obtained and used for Western blot experiments.

Statistical analysis. Statistical data were reported as means ± 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 NECA, CGS 21680, and GW 7647-induced vascular responses to antagonist (t-NAME, 14,15-EEZE, T0070907, ZM 241385, SCH 58261, AUDA, and t-AUCB). Differences were considered significant when P < 0.05. Further, densitometry of Western blot analysis (CYP2J5, CYP4A, PPARα, PPARγ, A1AR, and A2A AR) was expressed as means ± SE in arbitrary units. All the statistical analyses were performed using GraphPad Prism statistical package.

RESULTS

Expression of A2A AR, A1 AR, CYP2J5, CYP4A, PPARα, and PPARγ proteins in aortas from sEH−/− and sEH+/+ mice. Western blot analysis for A2A AR (~45 kDa) protein showed 31% more in sEH−/− than sEH+/+ mouse aorta (P < 0.05, Fig. 1A) whereas, A1 AR (~37 kDa) protein showed 30% less in sEH−/− than sEH+/+ mice (P < 0.05, Fig. 1B). The amount of CYP2J5 (~58 kDa) protein in sEH−/− was increased by 65% compared with sEH+/+ mouse aorta (P < 0.05, Fig. 2A). Further, the level of CYP4A (~50 kDa) protein in sEH−/− was increased by 60% compared with sEH+/+ mouse aorta (P < 0.05, Fig. 2B). Western blot analysis for PPARγ (~58 kDa)
While in sEH, protein showed 36% more in sEH/− than sEH/+ mouse aorta (P < 0.05, Fig. 3A), whereas PPARα (~52 kDa) protein showed 27% less in sEH/− than sEH/+ mice (P < 0.05, Fig. 3B).

CRC for ACh and the effect of nitric oxide inhibitor in sEH/− and sEH/+ mice. ACh caused a concentration (10⁻⁷–10⁻⁵ M)-dependent relaxation in both sEH/− and sEH/+, but the response was not significantly different (P > 0.05) between aortas from sEH/− and sEH/+ (Fig. 4). Also, L-NAME (100 µM) had altered vascular response significantly (P < 0.05) in both sEH/− (+3.32 ± 6.0% at 10⁻⁶ ACh) and sEH/+ (−3.4 ± 2.9% at 10⁻⁶ M ACh) compared with untreated sEH/− and sEH/+ mouse aortas (P < 0.05, Fig. 4). But, no significant difference was observed in concentration response curves between sEH/− and sEH/+ (P < 0.05, Fig. 4).

CRC for NECA with and without ZM 241385 or SCH 58261 in sEH/− and sEH/+ mice. NECA produced a concentration-dependent relaxation in sEH/− as opposed to contraction in sEH/+ (Fig. 5, A and B). For example, the response to 10⁻⁶ M NECA in sEH/− aorta was +12.94 ± 3.2% relaxation, while in sEH/+ had −5.35 ± 5.2% contraction (P < 0.05, Fig. 5, A and B). CRC in sEH/− vs. sEH+/+ for NECA (10⁻⁷–10⁻⁵ M, P < 0.05) were significantly different. ZM 241385 (1 µM), an A2A AR antagonist produced a change from NECA-induced relaxation to contraction in sEH/− (from +12.94 ± 3.2% to −22.42 ± 1.9 at 10⁻⁶ NECA, P < 0.05, Fig. 5A). No significant difference was found with ZM 241385 treatment between sEH/− and sEH/+ mice (P > 0.05, Fig. 5A). Another A2A AR antagonist, SCH 58261 produced a similar change from NECA-induced relaxation to contraction in sEH/− (from +12.94 ± 3.2% to −30.04 ± 4.2 at 10⁻⁶ NECA, P < 0.05, Fig. 5B). No significant difference was found between SCH 58261 treated sEH/− and SCH 58261 treated sEH/+ mice (P > 0.05, Fig. 5B).

CRC for CGS 21680 and the effects of nitric oxide/epoxyeicosatrienoic acid receptor-antagonist in sEH/− and sEH/+ mice. CGS 21680 produced a concentration-dependent relaxation (P < 0.05) in sEH/− compared with the contraction in sEH/+ mice (P < 0.05; Fig. 6). For example, at 10⁻⁶ M CGS 21680, the relaxation response was +37.4 ± 5.4% in sEH/− compared with +2.1 ± 2.8% in sEH/+ mice (P < 0.05; Fig. 6A). L-NAME (100 µM) did not significantly alter vascular responses in both the treated sEH/− (+30.28 ± 4.8% at 10⁻⁶ CGS 21680) and the control sEH/− (+37.4 ± 5.4%, P > 0.05, Fig. 6A) tissues. Also, no significant difference was observed in CRC between treated (L-NAME) and control

Fig. 1. Representative Western blots and densitometric analysis for A2A AR (~45 kDa; A) and A1AR (~37 kDa; B) proteins in aortas of sEH/− and sEH/+ mice. Values are expressed as means ± SE. *P < 0.05, sEH/+ compared with sEH/− aortas; n = 6.

Fig. 2. Representative Western blots and densitometric analysis for CYP2J5 (~58 kDa; A) and CYP4A (~50 kDa; B) proteins in aortas of sEH/− and sEH/+ mice. Values are expressed as means ± SE. *P < 0.05, sEH/+ compared with sEH/− aortas; n = 6.
sEH+/+ aorta (+5.1 ± 2.4 vs. +2.1 ± 2.8% at 10⁻⁶ CGS 21680, P < 0.05, Fig. 6A). Whereas, a significant blockade was found in the CRC in CGS 21680-induced relaxation with 14,15-EEZE (10 μM, a EETs receptor antagonist) compared with control in sEH⁻/⁻ aortas. At 10⁻⁶ M CGS 21680, 14,15-EEZE had changed the relaxation response into contraction in sEH⁻/⁻ (-7.1 ± 3.7%, Fig. 6B) aortas compared with controls (+37.46 ± 5.4%, P < 0.05; Fig. 6B). The CGS 21680-induced CRC unchanged among 14,15-EEZE-treated sEH+/+, 14,15-EEZE-treated sEH⁻/⁻, and control sEH+/+ tissues (P > 0.05, Fig. 6B).

Effects of sEH inhibitors on CGS 21680 CRC in sEH⁻/⁻ and sEH+/+ mice. AUDA (10 μM), a soluble epoxide hydrolase (sEH) inhibitor, reversed and produced a significantly higher relaxation (+45.33 ± 4.1%, P < 0.05, Fig. 7A) with CGS 21680 in sEH+/+ as opposed to its control (+2.1 ± 2.8%; Fig. 7A). In contrast, AUDA had no significant effect on CGS 21680 CRC in treated and untreated sEH⁻/⁻ tissues (P > 0.05, Fig. 7A). Also, no significant differences were observed in CGS 21680 CRC among sEH+/+ and sEH⁻/⁻ tissues treated with AUDA and untreated EH⁻/⁻ (P > 0.05, Fig. 7A).

DISCUSSION

The sEH⁻/⁻ mice show an increase in aortic A₂A AR, CYP2J, and PPARα protein expressions, and a decrease in A₁ AR, PPARα proteins compared with sEH+/+ mice (Figs. 1–3). The relationship between sEH and adenosine-induced vascular responses in mice is not known. Therefore, this study was designed to investigate the role of A₂A, A₁ ARs, CYP-epoxygenases, PPARα, and PPARγ in sEH+/+ and sEH⁻/⁻ mice. Our data demonstrate that 1) ACh-induced vascular relaxation
CGS 21680 enhanced dose-dependent relaxation in sEH mice. While the earlier studies showed the differential renal sEH gene expression in prehypertensive, hypertensive, and spontaneously hypertensive rats (49), and mice lacking A2A AR have hypertensive characteristics (22). Therefore, there is a need to identify the possible targets and develop novel pharmacological agents to treat vascular deregulation in patients in the long run, who have allelic variants that may possibly act similar to our gene-manipulated mice (A2A AR−/−, sEH−/−), which may be involved in the regulation of blood pressure and vascular tone.

ACh data between sEH+/+ and sEH−/− suggest that there is no relationship between ACh and the presence or absence of

was not different between sEH−/− and sEH+/+ mice, and L-NAME was able to block ACh-induced vascular relaxation equally in both sEH−/− and sEH+/+ mice; 2) A2A AR modulates nitric oxide (NO)-independent vascular relaxation in sEH−/− mice compared with sEH+/+; 3) A2A AR-mediated relaxation was blocked by EET receptor antagonist, but not with NO inhibitor; 4) sEH inhibitors (AUDA and t-AUCB) reversed the CGS 21680-mediated vascular response to enhanced relaxation in sEH+/+, but ineffective in sEH−/− mice; 5) CGS 21680 enhanced dose-dependent relaxation in sEH−/− mice, whereas GW 7647 (PPARγ-agonist) reduced relaxation significantly, and finally 6) T0070907 (PPARγ-antagonist) significantly inhibited the CGS 21680-enhanced vascular relaxation in sEH−/− mice.

Genetic polymorphisms in CYP-epoxygenases have been observed in different populations, which affect cardiovascular function, including hypertension (10, 12, 20, 23, 54, 55, 57, 66). Genetic variation in sEH and CYP4A was also found in human population with risk of coronary heart disease, ischemic stroke, restenosis, diabetes heart, heart failure, ischemic stroke in white Europeans, Chinese populations, and in the African American population with hypertension (2, 10, 21, 24, 30, 66). As the earlier studies showed the differential renal sEH gene expression in prehypertensive, hypertensive, and spontaneously hypertensive rats (49), and mice lacking A2A AR have hypertensive characteristics (22). Therefore, there is a need to identify the possible targets and develop novel pharmacological agents to treat vascular deregulation in patients in the long run, who have allelic variants that may possibly act similar to our gene-manipulated mice (A2A AR−/−, sEH−/−), which may be involved in the regulation of blood pressure and vascular tone.

ACh data between sEH+/+ and sEH−/− suggest that there is no relationship between ACh and the presence or absence of
During exercise in humans, rats, and rabbits (43, 44, 50, 58), sEH (Fig. 4). Also, L-NAME, a nitric oxide synthase inhibitor completely blocked ACh-induced vascular response equally in both sEH+/+ and sEH−/− mice (Fig. 4). These data also suggest that the presence or absence of sEH does not matter in ACh-induced vascular response in both sEH+/+ and sEH−/− mice (Fig. 4). Similarly, Zhang et al. (65) reported that the inhibition of sEH ameliorates endothelial dysfunction and effects in the db/db mice, which is independent of NO, but dependent on CYP-epoxygenase-derived metabolites. Other studies have also shown that nitric oxide synthase inhibition does not affect the increase of blood flow in forearm or leg during exercise in humans, rats, and rabbits (43, 44, 50, 58). But, during exercise, adenosine originates from skeletal muscle fibers and acts on A2A AR to evoke vasodilatation independent of NO (45). Our own studies have shown that adenosine-

![Fig 7](http://ajpregu.physiology.org/)

![Fig 8](http://ajpregu.physiology.org/)
induced mouse aortic relaxation through A2A AR is independent of NO and COX (36–38).

The sEH enzyme metabolizes EET that serve as substrates for the sEH (31, 40). The conversion of EETs into the corresponding dihydroxyeicosatetraenoic acids (DHETs) results in loss of beneficial effects (29, 52, 62). The conversion of epoxides to diols by sEH diminishes the beneficial cardiovascular properties of these epoxyeicosanoids. Inhibition of sEH causes EETs to accumulate and be retained for longer periods after they are formed (9). Some reports provide further evidence that sEH inhibition may be an effective approach for the treatment of hypertension and diseases associated with vascular inflammation (7, 8, 18, 47, 61). Also, the targeted disruption of the sEH gene in male mice lowers systolic blood pressure (51). Recently, we found that there is a possible link between the upregulation of A2A AR, CYP-epoxygenases, and downregulation of sEH with adenosine-induced relaxation in high-salt diet-fed WT mice (C57BL/6J) and eNOS−/− (C57BL/6J background) compared with low-salt diet-fed mice (38, 39). Also, we found that there is a possible link between the downregulation of A2A AR, CYP-epoxygenases and upregulation of sEH with NECA-induced contraction in low-salt diet-fed WT mice and eNOS−/− (C57BL/6J background) compared with high-salt diet-fed mice (38, 39). The current study shows that in the absence of sEH, there is an increase in aortic A2A AR, CYP2J5, PPARγ, and a decrease in A1 AR, PPARα proteins compared with WT mice. Therefore, we hypothesize that, in the absence of sEH, adenosine induces vascular relaxation through A2A AR via CYP-epoxygenases and PPARγ, whereas in the presence of sEH, adenosine induces vascular contraction through PPARα.

NECA (nonselective adenosine agonist) induces significant relaxation in sEH−/− compared to sEH+/+ mice (Fig. 5), suggesting that sEH deletion enhances NECA-induced vascular relaxation. Our data show that NECA-induced vascular response in aortas of sEH−/− mice is similar to that from our earlier reports in which we found that high-salt diet-fed WT (C57BL/6J) mice had an enhanced NECA-induced relaxation with upregulation of A2A AR, CYP2J-epoxygenase and downregulation of sEH, whereas, NECA-induced vascular response in aortas of sEH+/+ mice is similar to that found in our earlier reports in which low-salt diet-fed WT (C57BL/6J) mice had an enhanced NECA-induced contraction with downregulation of A2A AR, CYP2J-epoxygenase and upregulation of sEH (37, 38). Therefore, in the present study, we used specific A2A AR antagonists (ZM-241385 and SCH-58261) to rule out the involvement of other adenosine receptors (A1, A2B, A3). Both ZM 241385 and SCH-58261 (A2A AR-antagonists) blocked completely the NECA-induced vascular relaxation in sEH−/− compared with untreated mice (Fig. 5, A and B). We also confirmed the involvement of A2A AR with the use of a highly selective A2A AR agonist (CGS 21680) in sEH+/+ and sEH−/− mouse aortas. A highly robust CGS 21680-induced vascular relaxation was observed in sEH−/− compared with sEH+/+ mice (Fig. 6). Also, in the present study, we found a significant upregulation of A2A AR protein in sEH−/− compared with sEH+/+ mice (Fig. 1A), whereas a significant downregulation of A1 AR protein in sEH−/− compared with sEH+/+ mice (Fig. 1B). These data suggest that A2A AR is the only adenosine receptor involved in enhancing adenosine-induced relaxation in sEH−/− compared with sEH+/+ mouse aortas.

In the present study, we also tested whether A2A AR-induced vascular relaxation is NO-dependent or not. No significant changes were found between l-NAME-treated and untreated sEH−/− and sEH+/+ mouse aortas with CGS 21680-induced vascular response (Fig. 6A). These data suggest that there is no role for NO in adenosine-induced vascular response through A2A AR in sEH−/− and sEH+/+ mice. These data confirm our earlier reports from this laboratory in which l-NAME and indomethacin (COX inhibitor) were unable to block aortic relaxation in A2A AR+/+ compared with A2A AR−/− mice, and in high-salt diet-fed compared with low-salt diet-fed C57BL/6J mice (36–39). Also, Ray and Marshall (45) reported that during exercise, skeletal muscle fibers release adenosine, which is independent of NO.

In this study, NECA- and CGS 21680-induced relaxation in sEH−/− mouse aortas (Figs. 5–8) suggests that this relaxation depends on A2A AR via CYP-epoxygenases leading to EETs formation. Therefore, we were able to block CGS 21680-induced vascular relaxation with 14,15-EEZE (EET receptor antagonist) in sEH−/− mouse aortas (Fig. 6B). Similarly, the EDHF response in bovine coronary arteries was inhibited by the EET antagonist, 14,15-epoxyeicosatrienoic acid (Z)-enicoic acid (14). Recently, a report from our laboratory also showed that in A2A AR+/+ mouse aorta, the adenosine-induced vascular relaxation was inhibited by the EET antagonist, 14,15-epoxyeicosatrienoic acid (36). In the present study, we also found an upregulation of CYP2J5 proteins in sEH−/− compared with sEH+/+ mouse aortas (Fig. 2A). Our data suggest that the endothelium-dependent NECA- and CGS-21680-induced vascular relaxation is mediated through EETs possibly by increased CYP2J-epoxygenase in sEH−/− compared sEH+/+ mouse aortas. The current study further confirmed that the adenosine-induced vascular relaxation through A2A AR is dependent on CYP-epoxygenases, including CYP2J-epoxygenase, but not NO or COX.

We also demonstrated that the treatment with 12-(3-adaman-1-yl-ureido) dodecanoic acid (AUDA) or trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (t-AUCB), inhibitors of the sEH enzyme have reversed the CGS 21680-induced weak vascular response into a strong vascular relaxation in sEH+/+ compared with controls (Fig. 7, A and B), while, no significant change was observed with AUDA or t-AUCB-treated and untreated sEH−/− mice (Fig. 7, A and B). These data suggest that, in the presence of sEH, AUDA or t-AUCB effectively block the endogenous sEH enzyme activity. Therefore, we observed complete reversal of CGS 21680-induced vascular response in AUDA- or t-AUCB-treated sEH+/+ mouse aortas compared with controls. Also, it is obvious that, no significant change was observed with CGS 21680-induced vascular response between AUDA or t-AUCB-treated and untreated sEH−/− mice, because these mice do not have endogenous sEH enzyme activity. Use of sEH enzyme inhibitors or deleting the sEH gene increases endogenous EETs by decreasing the metabolic conversion of EETs into less active DHETs. If the mechanism by which sEH inhibitors (AUDA or t-AUCB) or deletion of sEH gene enhance CGS 21680-induced vascular relaxation occurs by increasing endogenous EET levels, then it would be expected that inhibiting EET synthesis would attenuate the CGS 21680-induced vascular response in sEH−/− mice. As expected, pretreatment of sEH−/− mouse aorta with the EETs antagonist 14,15-EEZE did...
alter significantly CGS 21680-induced vascular response from relaxation to contraction (Fig. 6B). These data suggest that in absence or blocking of sEH, enhanced CGS 21680-induced vascular relaxation is mostly likely due to an increase EET levels. Liu et al. (26) suggests that selective sEH inhibitors will potentiate the anti-inflammatory effect in the endothelial cells, presumably by increasing the retention of 11,12- and 14,15-EET so that PPARγ activation is prolonged. Acute activation of PPARγ leads to endothelium-dependent aortic relaxation in nondiabetic (+dib/m) mice (48). Therefore, we found upregulation of PPARγ and down regulation of PPARα with A2AR AR enhanced relaxation in sEH−/− compared with sEH+/+ mice (Fig. 3, A and B). The PPAR expression data are also supported by the functional data, in which PPARα agonist dose-dependent vascular response in sEH−/− was not significantly different compared with sEH+/+ mice (Fig. 8A), whereas CGS 21680 dose-dependent vascular response in sEH−/− was significantly different compared with sEH+/+ mice (Fig. 8B). There was no significant difference found between CGS 21680-induced vascular response in sEH+/+ mice compared with PPARα-agonist-induced response in both sEH−/− and sEH+/+ mice (Fig. 8, A and B), suggesting that the vascular contraction in sEH+/+ mouse aorta is possible due to PPARα activity (Figs. 3B and 8A). Moreover, PPARγ antagonist was able to block significantly the CGS 21680-induced vascular relaxation (Fig. 8C), and there was no significant difference found between CGS 21680-induced vascular response in sEH+/+ mice compared with CGS 21680 + PPARγ antagonist response in both sEH−/− and sEH+/+ treated mice (Fig. 8C). These data suggest a possible role for PPARγ in CGS 21680-induced vascular relaxation in the absence of sEH compared with the presence of sEH in mice (Figs. 3A and 8C). Also, the data suggest a possible role of PPARα in vascular contraction (Figs. 3B and 8A).

Surprisingly, we found an upregulation of CYP4A (Fig. 2B), a vasoconstrictor enzyme in sEH−/− mouse aorta compared with sEH+/+. The reason behind the upregulation of CYP4A in the absence of sEH may be due to a compensatory response to maintain vascular tone, as well as the blood pressure in sEH knockout mouse. Similar upregulation of CYP4A in kidneys of Ephx2 gene-disrupted mice has been reported as a compensatory mechanism to maintain blood pressure regulation (27).

The present data suggest that there is a relationship between A2AR AR-enhanced vascular relaxation and lack of sEH, and there is relationship between the presence of sEH and adenosine-induced vasoconstriction or less relaxation through upregulation of A1 AR. The signaling mechanism may be involved by the activation of A2AR AR, functional endothelium to activate CYP2J-epoxygenases to generate more EETs than DHETs, downregulation of A1 AR, PPARα, and upregulation of A2AR AR, PPARγ, leading to NECA or CGS 21680-enhanced vascular relaxation in sEH−/− mouse aorta. In contrast, the contraction or less relaxation with NECA or CGS 21680 in sEH+/+ mice provides evidence that there is a possible link among the less availability of A2AR AR, less functional endothelium to activate CYP2J-epoxygenases, less generation of EETs than DHETs, and upregulation of A1 AR and PPARα. Therefore, we conclude that upregulation of CYP-epoxygenases, A2AR AR, and PPARγ and downregulation of A1 AR and PPARα protein expression contribute to NECA- or CGS 21680-induced dilation in sEH−/− mouse aorta, while the upregulation of A1 AR and PPARα and downregulation of CYP-epoxygenases, A2AR AR, and PPARγ protein expression contribute to NECA or CGS 21680-induced vasoconstriction or less relaxation in sEH+/+ mouse aorta.

PERSPECTIVES AND SIGNIFICANCES

These findings suggest that the relationship among A2AR AR, CYP-epoxygenases, arachidonic acid-derived metabolites, and PPARγ in vasodilation, while a relationship among A1 AR, sEH, and PPARα in vasoconstriction may have clinical implication in the regulation of vascular tone and regulation of blood pressure. Any deregulation in these pathways or slight allelic variations may possibly lead to hypertension and coronary artery disease in humans. Future studies are necessary to identify the possible targets and develop novel pharmacological agents to treat vascular deregulation in patients in the long run, who have allelic variants that may possibly act similar to our gene-manipulated mice (A2AR AR−−, sEH−−), which may play a role in the regulation of vascular tone and ultimately blood pressure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: M.A.N. conception and design of research; M.A.N. and I.P. performed experiments; M.A.N. analyzed data; M.A.N. interpreted results of experiments; M.A.N. prepared figures; M.A.N. drafted manuscript; M.A.N., I.P., S.J.M., C.M., J.R.F., and D.C.Z. approved final version of manuscript.

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