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Reduced Coronary Reactive Hyperemia in Mice was Reversed by the Soluble Epoxide Hydrolase Inhibitor (*t*-AUCB): Role of Adenosine A_{2A} Receptor and Plasma Oxylipins

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Abstract

Coronary reactive hyperemia (CRH) protects the heart against ischemia. Adenosine A_{2A}AR–deficient ($A_{2A}AR^{-/-}$) mice have increased expression of soluble epoxide hydrolase (sEH); the enzyme responsible for breaking down the cardioprotective epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatrienoic acids (DHETs). she–inhibition enhances CRH, increases EETs, and modulates oxylipin profiles. We investigated the changes of oxylipins and their impact on CRH in $A_{2A}AR^{-/-}$ and wild type (WT) mice. We hypothesized that the attenuated CRH in $A_{2A}AR^{-/-}$ mice is mediated by changes in oxylipin profiles, and that it can be reversed by either sEH– or ω - hydroxylases–inhibition. Compared to WT mice, $A_{2A}AR^{-/-}$ mice had attenuated CRH and changed oxylipin profiles, which were consistent between plasma and heart perfusate samples, including decreased EET/DHET ratios, and increased hydroxyeicosatetraenoic acids (HETEs).

Plasma oxylipns in $A_{2A}AR^{-/-}$ mice indicated an increased proinflammatory state including increased ω-terminal HETEs, decreased epoxyoctadecaenoic / dihydroxyoctadecaenoic acids (EpOMEs/DiHOMEs) ratios, increased 9-hydroxyoctadecadienoic acid, and increased prostanoids. Inhibition of either sEH or ω-hydroxylases reversed the reduced CRH in $A_{2A}AR^{-/-}$ mice. In WT and sEH $^{-/-}$ mice, blocking A_{2A}AR decreased CRH. These data demonstrate that A_{2A}AR–deletion

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was associated with changes in oxylipin profiles, which may contribute to the attenuated CRH. Also, inhibition of sEH and ω-hydroxylases reversed the reduction in CRH.

Keywords

Adenosine A_{2A} receptor; coronary reactive hyperemia; ω-hydroxylases; soluble epoxid; hydrolase; heart perfusate oxylipins; plasma oxylipins

Introduction

Reactive hyperemia (RH) is a physiologic phenomenon whose function is to rapidly restore blood perfusion by increasing blood flow to an ischemic organ or tissue [1]. The myocardium is perpetually active and responds to coronary occlusion through the same mechanism: coronary reactive hyperemia (CRH) [2]. Ischemia is inherently harmful, and the increased blood flow associated with CRH seems to serve a protective role by increasing blood supply to the deprived cardiac tissue [1, 3]. The importance of CRH is reiterated by the observation that several cardiovascular pathologic conditions affecting the coronary circulation, such as the metabolic syndrome [4], cardiac hypertrophy [5], coronary artery disease, and congestive heart failure [6] have documented abnormalities in CRH. In addition to a number of mediators, including hydrogen peroxide (H_2O_2) [5], nitric oxide (NO) [5], and Katp channels [5], adenosine mediates CRH through its receptor subtype A_{2A}AR [5, 7, 8]. Our lab has recently reported that several arachidonic- and linoleic-acid derived oxylipins, such as EETs, DHETs, EpOMEs, DiHOMEs, mid-chain HETEs, and hydroxyoctadecadienoic acids (HODEs), could impact CRH [3, 9-11]. Arachidonic and linoleic-acids are two important ω-6 PUFAs (polyunsaturated fatty acids), which can be oxidized to oxylipins known to mediate myriad of physiologic effects [12]. Cytochrome P450 epoxygenases generate epoxyeicosatrienoic acids (EETs) from arachidonic acid (AA) by epoxidation, whereas Cytochrome P450 ω-hydroxylases hydroxylate AA to form ω-terminal HETEs [3]. EETs have protective functions in the cardiovascular system including vasodilation through hyperpolarization [13, 14]. A cardioprotective effect against ischemia/reperfusion injury was observed by the increased generation of EETs in cardiomyocytes in mice [15]. Soluble epoxide hydrolase (sEH) breaks EETs down to form DHETs, which are less active. To investigate the effects of EETs in experimental studies, sEH is often utilized either by pharmacologic inhibition [9, 16], genetic deletion [9, 15, 17], or over-expression [3, 18]. In the isolated mouse heart model, sEH inhibition was associated with enhanced CRH and increased EET/DHET ratio [9], whereas endothelial over-expression of sEH attenuated CRH [3], decreased EET/DHET ratio, and impaired endothelium-dependent vasodilation in mouse cerebral circulation [18]. Of the ω-terminal HETEs, 20-HET, which is a potent vasoconstrictor, is produced by CYP4A from arachidonic acid [19]. 20-HETE promotes hypertension, vasoconstriction, and vascular dysfunction [20, 21]. Like arachidonic acid, linoleic acid is converted to vasoactive oxylipins, such as EpOMEs and HODEs. At physiological levels, EpOMEs were protective against hypoxia injury [22, 23]. One of the two isomers, 12,13-EpOME, was protective for rabbits' renal proximal tubular cell cultures against hypoxia/reoxygenation injury [24]. sEH metabolizes EpOMEs to

DiHOMEs; the latter have deleterious effects in the heart and are cytotoxic to cells in tissue culture at high concentrations [25].

Adenosine is generally cytoprotective; it is an endogenous nucleoside produced in response to stressful conditions, such as inflammation and ischemia [26]. Adenosine's cardioprotective effects are mediated by its receptor subtypes A_{2A}AR and A_{2B}AR [27]; A_{2A}AR is important in mediating the vasodilatory effect of adenosine [28], including coronary reactive hyperemia in response to ischemia in dogs [8]. Adenosine inhibits platelet aggregation through its A_{2A}AR, which is expressed on murine and human platelets [29]. A genetic variant in adenosine A_{2A}AR in humans, ADORA2A TT variant, predisposes to increased changes in mean and peak systolic blood pressure in response to caffeine [30]. Moreover, a single-nucleotide polymorphism variant of A_{2A}AR, rs4822489, was linked to the severity of chronic heart failure in a group of Chinese people [27]. EETs mediate A_{2A}AR–induced vasodilation in rat arcuate arteries [31]. The A_{2A}AR–EET pathway is further supported by the finding that A_{2A}AR–activation results in increased EETs' release in isolated perfused kidney [32]. Our lab demonstrated that sEH expression was increased in $A_{2A}AR^{-/-}$ (A_{2A}AR-null) mice compared to $A_{2A}AR^{+/+}$ (wild type) mice [33]. Increased sEH expression was associated with attenuated CRH [3], and decreased EET/DHET ratio [18].

The effect of sEH– and ω-hydroxylases–inhibition on CRH in $A_{2A}AR^{-/-}$ mice in response to ischemia, and the changes in oxylipin profiles in plasma and heart perfusate associated with genetic deletion of A_{2A}AR in $A_{2A}AR^{-/-}$ mice have not been investigated. We hypothesized that the attenuated CRH in $A_{2A}AR^{-/-}$ mice is partially mediated by changes in oxylipin profiles, and that it can be reversed either by soluble epoxide hydrolase– or ω-hydroxylases–inhibition.

Methods

Animals

The mice used in this paper, sEH $^{-/-}$, $A_{2A}AR^{-/-}$, and wild type (WT), were of the C57BL/6 genetic background. $A_{2A}AR^{-/-}$ mice (initially from C. Ledent, Universite Libre de Bruxelles, Brussels, Belgium) were obtained from Dr. Stephen Tilley, UNC Chapel Hill. $A_{2A}AR^{-/-}$ mice were backcrossed 12 generations to the C57BL/6 background as previously reported [34]. The breeder pairs of $A_{2A}AR^{-/-}$ mice were generously provided by Dr. Jamal Mustafa (WVU). The generation of sEH $^{-/-}$ mice was described by Sinal et al. [35]. sEH $^{-/-}$ mice were provided by Dr. Darryl Zeldin, National Institute of Environmental Health Sciences/National Institutes of Health (NIH). Animal care and experimentation protocols were approved and performed in conformity with the West Virginia University Institutional Animal Care and Use Committee and were in compliance with the guidelines of the NIH's *Guide for the Care and Use of Laboratory Animals*. Equal ratios of male and female mice, age 16 –18 weeks, were used in this study. Mice were cared for in cages with a 12:12 h light-dark cycle and allowed access to standard chow (Cat #2018, Envigo, Indianapolis, IN) and water. Diet 2018 was used and contained 6.2% fat by weight, including 1.2% oleic %, 0.3% linolenic, 0.2% stearic, 0.7% palmitic, and 3.1% linoleic acids.

Langendorff-Perfused Heart Preparation

A constant pressure mode of 80 mmHg was used in the Langendorff isolated heart perfusion as previously described [3, 9-11]. Briefly, $sEH^{-/-}$, $A_{2A}AR^{-/-}$, and wild-type (WT) mice (16–18 wks.) were euthanized using 100 mg/kg body weight sodium pentobarbital intra-peritoneally. Hearts were excised and instantly placed into ice-cold Krebs-Henseleit buffer containing (in mM) 1.2 KH_2PO_4, 11.0 glucose, 4.7 KCl, 119.0 NaCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 2.0 pyruvate, 22.0 $NaHCO_3$, 0.5 EDTA, and heparin (5 U/mL). The lungs and surrounding tissues around the heart were then removed, the aorta cannulated, and the heart continuously perfused with warm (37°C) buffer bubbled with 95% O_2. Measurement of left ventricular developed pressure (LVDP) and heart rate (HR) was provided by a water-filled balloon placed into the left ventricle and connected to a pressure transducer. Coronary flow (CF) was measured by a flow transducer with an ultrasonic flow probe (Transonic Systems, Ithaca, NY). Data acquisition and recording was done by a Power–Lab Chart (AD Instruments, Colorado Springs, CO). Hearts were left to stabilize for 30–40 min before experimentation. Hearts with CF increase by less than two-fold in response to a 15-second total occlusion, as well as hearts with LVDP <80 mmHg or persistent arrhythmias were excluded from analysis.

Coronary Reactive Hyperemic Response

Upon stabilization, the valve above the cannulated heart was closed for 15 seconds to produce CRH. Pre- and post-CRH repayment volume (RV), CF, repayment duration (RD), LVDP, and HR were analyzed for all hearts. A microinjection pump (Harvard Apparatus, Holliston, MA) was used to infuse the investigational drugs for 15 minutes. A second CRH was produced and the same data collected again for the same heart. Drugs were dissolved in dimethyl sulfoxide (DMSO) and brought to the target concentration by adding PBS (Phosphate buffered saline). DMSO's final concentration was < 0.1%. Drugs' infusion rate was 1% of CF. The final concentrations, after standardization of dose (0.01, 0.1, 1, & 10 μM) response for the used drugs were 0.1 μM SCH-58261, selective A_{2A}AR-antagonist, and 1 μM DDMS (dibromo-dodecenyl-methylsulfimide, CYP4A-blocker), and 10 μM *t*-AUCB (*trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (a selective sEH-inhibitor, University of California, Davis) [36]. These concentrations were less than or equal to concentrations used in earlier studies: *t*-AUCB, 10 μM; [3, 9, 10, 37], DDMS, 1 μM [38].

Effect of SCH-58261 (A_{2A}AR-antagonist) on CRH Response in WT and $sEH^{-/-}$ Mice

The hearts from WT and $sEH^{-/-}$ mice were exposed to 15 seconds of occlusion. CRH parameters (RV, RD, baseline CF, HR, and LVDP) were recorded and averaged. SCH-58261's final concentration was 0.1 μM and was injected for 15 min. Then, another CRH followed and the same recordings taken again.

Effect of the sEH-inhibitor, *t*-AUCB, on CRH Response in $A_{2A}AR^{-/-}$ Mice

Similar to the previous section, but using $A_{2A}AR^{-/-}$ mice, the sEH-inhibitor, *t-AUCB,* was infused at 10 μM for 15 min. CRHs before and after *t*-AUCB infusion were analyzed and compared.

Effect of DDMS (ω-hydroxylases-inhibitor) on CRH Response in $A_{2A}AR^{-/-}$ Mice

As described above, CRH in $A_{2A}AR^{-/-}$ mice before and after the ω-hydroxylases-inhibitor (DDMS) treatment (at a 1 μM for 15 min) were compared.

LC–MS/MS Oxylipin Analysis

Oxylipins (EETs and their metabolites DHETs, mid-chain and ω-terminal HETEs, EpOMEs and their metabolites DiHOMEs, HODEs, and prostanoids) were measured in plasma and heart perfusate samples of $A_{2A}AR^{-/-}$ mice by liquid chromatography, tandem mass spectroscopy (LC-MS/MS) as described previously [9, 39]. Briefly, we collected the heart perfusate at baseline and after occlusion. For the plasma samples, submandibular blood collection in the amount of 0.5 -0.7 mL was carried out by a trained lab staff. Each mouse was restrained and a puncture in its cheek was made with a 5.0 mm lancet. The blood was then collected in 1.5 mL–Eppendorf tubes placed on ice, gently shaken, and allowed to settle for 1 hour. After the desired amount was collected, gauze was placed on the mouse's cheek to ensure that the bleeding had stopped. Each blood sample was then centrifuged at 10,000 g for 10 min, and the supernatant was collected. Both heart perfusate and plasma samples were stored at −80°C until processing. The reader is referred to our published paper [9] for details on the quantification procedure.

Statistical and Data Analyses

The reader is referred to our paper [9] for definitions and calculations of flow debt and repayment volume. Values are presented as means ± standard error; *n* denotes number of animals used in experiments and analysis. Data from two independent groups were analyzed by Student *t*-test, whereas data from more than two groups were analyzed using two-way ANOVA. Differences were considered statistically significant when $P < 0.05$.

Results

CRH Response

Effect of SCH-58261 ($A_{2A}AR$–antagonist) on CRH Response in WT and $sEH^{-/-}$ Mice—The selective $A_{2A}AR$–antagonist, SCH-58261, decreased CRH in both WT and $sEH^{-/-}$ mice. Repayment volume (RV) was decreased in both WT mice (from 5.0 ± 0.6 to 2.3 ± 0.3 mL/g; $P < 0.05$) and $sEH^{-/-}$ mice (from 7.4 ± 0.7 to 3.4 ± 0.4 mL/g; $P < 0.05$, Fig 1A). Repayment/debt (R/D) ratio was also decreased in WT (from 1.3 ± 0.2 to 0.6 ± 0.1; $P < 0.05$) and $sEH^{-/-}$ mice (from 1.8 ± 0.2 to 1.3 ± 0.1; $P < 0.05$, Fig 1B). Repayment duration (RD) was decreased in WT (from 1.4 ± 0.3 to 0.4 ± 0.1 min; $P < 0.05$) and $sEH^{-/-}$ mice (from 2.1 ± 0.3 to 0.9 ± 0.2 min; $P < 0.05$, Fig 1C). Blocking $A_{2A}AR$ by SCH-58261 decreased baseline CF in WT (from 15.9 ± 0.7 to 13.0 ± 0.6 mL/min/g) and $sEH^{-/-}$ mice (from 15.0 ± 0.7 to 10.8 ± 0.8 mL/min/g; $P < 0.05$, Fig 1D). Comparing the untreated groups (WT vs. $sEH^{-/-}$ mice) demonstrated significantly increased RV, R/D ratio, and RD in $sEH^{-/-}$ compared to WT mice (Fig 1A-C). There was no significant difference between SCH-58261–treated WT and SCH-58261–treated $sEH^{-/-}$ mice in the above– mentioned parameters based on the 2-way ANOVA Analyses of Interaction between the 2 variables: mouse genotype and drug (SCH-58261) treatment. LVPD and HR were not different

between and within the two groups ($P > 0.05$; Fig 1E-F). Due to the lack of sex difference in CRH response, we used equal ratios of male and female mice from each strain for the remainder of the study.

Effect of $A_{2A}AR$–deletion on CRH Response—Mice with deleted $A_{2A}AR$ ($A_{2A}AR^{-/-}$) had attenuated CRH compared to WT mice. Compared to WT mice, $A_{2A}AR^{-/-}$ mice had decreased repayment volume by 25% (6.1 ± 0.5 and 4.9 ± 0.2 mL/g, respectively; $P < 0.05$, Fig 2A), decreased repayment/debt ratio (2.5 ± 0.2 and 1.9 ± 0.2, respectively; $P < 0.05$; Fig 2B), and decreased repayment duration (2.5 ± 0.3 and 1.2 ± 0.1 min, respectively; $P < 0.05$; Fig 2C). There was no statistically significant difference in LVPD, baseline CF, and HR between the two groups ($P > 0.05$; Fig 2D-F). Time-matched control experiments with WT mouse hearts, employing three consecutive inductions of CRH, showed no change in the CRH response and no difference in baseline heart functions, including LVDP, baseline CF, and HR (data not shown).

Effect of *t*-AUCB (sEH-inhibitor) on CRH Response in $A_{2A}AR^{-/-}$ Mice—The sEH-inhibitor, *t*-AUCB, enhanced CRH in $A_{2A}AR^{-/-}$ mice. Repayment volume was increased by 22% (from 5.8 ± 0.5 to 7.1 ± 0.8 mL/g; $P < 0.05$, Fig 3A), repayment/debt ratio from 1.6 ± 0.1 to 2.0 ± 0.2 ($P < 0.05$, Fig 3B), and repayment duration from 1.6 ± 0.2 to 2.3 ± 0.3 min ($P < 0.05$, Fig 3C). There was no significant difference between *t*-AUCB–treated and untreated $A_{2A}AR^{-/-}$ mice in baseline CF, LVPD, and HR ($P > 0.05$; Fig 3D-F).

Effect of DDMS (ω-hydroxylases-inhibitor) on CRH Response—DDMS enhanced CRH in $A_{2A}AR^{-/-}$ mice. Repayment volume was increased from 7.8 ± 0.7 to 11.8 ± 0.7 mL/g ($P < 0.05$, Fig 4A), repayment/debt ratio from 2.0 ± 0.2 to 3.0 ± 0.4 min ($P < 0.05$, Fig 4B), and repayment duration from 2.8 ± 0.6 to 4.6 ± 0.7 min ($P < 0.05$, Fig 4C). Baseline CF, LVPD, and HR were not different between the two groups ($P > 0.05$; Fig 4D-F).

Oxylipin Analysis of Heart Perfusate and Plasma in WT and $A_{2A}AR^{-/-}$ Mice—LC–MS/MS was used to determine oxylipin levels in heart perfusate and plasma samples.

EETs and DHETs: we were able to detect 11,12-DHET and 14,15-DHET in the heart perfusate, which were increased in $A_{2A}AR^{-/-}$ vs. WT mice at baseline and post-ischemia (Fig 5). However, only 11,12-DHET's increase vs. WT was statistically significant ($P < 0.05$; Fig 5A), whereas 14,15-DHET was not ($P > 0.05$; Fig 5B).

In plasma samples, 8,9-, 11,12-, and 14,15-EETs and their corresponding metabolites (8,9-, 11,12-, and 14,15-DHETs) were detected. The measured EETs (8,9-, 11,12-, and 14,15-EETs) were not significantly different between WT and $A_{2A}AR^{-/-}$ mice, ($P > 0.05$; Fig 6A-C). All measured DHETs were increased in $A_{2A}AR^{-/-}$ compared to WT mice ($P < 0.05$; Fig 6D-F). As a result, 8,9-, 11,12-, and 14,15-EET/DHET ratios decreased in $A_{2A}AR^{-/-}$ compared to WT mice ($P < 0.05$; Fig 6G-I).

Mid-chain HETEs: in heart perfusate, 5-, 11-, 12-, and 15-HETEs were detected in WT and $A_{2A}AR^{-/-}$ mice. Baseline and post-ischemic levels of 5-, 11-, 12-, and 15-HETE were increased in $A_{2A}AR^{-/-}$ compared to WT mice, and was significant for 11-, 12-, and 15-

HETEs ($P < 0.05$; Fig 7B-D), but not for 5-HETE ($P > 0.05$; Fig 7A). In plasma, the same mid-chain HETEs were detected. However, only 5-, 11-, and 15-HETEs were increased in $A_{2A}AR^{-/-}$ compared to WT mice ($P < 0.05$; Fig 8A, B and D), whereas 12-HETE was not different between the two groups ($P > 0.05$; Fig 8C).

ω–Terminal HETEs were detected only in plasma samples of WT and $A_{2A}AR^{-/-}$ mice. Both 19-and 20-HETE were increased in $A_{2A}AR^{-/-}$ compared to WT mice, but it was statistically significant in 20-HETE ($P < 0.05$; Fig 9B), not in 19-HETE ($P > 0.05$; Fig 9A)

Prostanoids: 6-keto-prostaglandin (PG)-$F_{1\alpha}$, PG-$F_{2\alpha}$, PG-D_2, PG-E_2, thromboxane B2 ($T\times B_2$) were detected in the plasma samples (Fig 10). These prostanoids were increased in $A_{2A}AR^{-/}$compared to WT mice, but were statistically significant only for PG-$F_{2\alpha}$, PG-E_2, and $T\times B_2$ ($P < 0$ 05; Fig 10B, C and E)

EpOMEs and DiHOMEs: these epoxides of linoleic acid were detected only in plasma samples. Compared to WT, $A_{2A}AR^{-/-}$ mice had similar levels of 9,10- and 12,13-EpOMEs ($P > 0.05$; Fig 11A and D), increased 9,10- and 12,13-DiHOMEs ($P < 0.05$; Fig 11B and E), and as a result, decreased 9,10- and 12,13- EpOME/DiHOME ratios ($P < 0.05$; Fig 11C and F).

HODEs: the two hydroxylated metabolites of LA, 9- and 13-HODEs (Fig 12), were detected only in plasma samples. 9-HODE, but not 13-HODE, was increased in $A_{2A}AR^{-/-}$ compared to WT mice ($P < 0.05$; Fig 12B).

The observed results and their possible impact on CRH were summarized into a proposed schematic diagram (Fig. 15).

Table 1 lists the statistical mean values and the standard error of the mean (SEM) for the measured plasma oxylipins for the two groups of mice. Also, the observed results and their possible impact on CRH were summarized into a proposed schematic diagram (Fig. 15).

Discussion

This study demonstrated that deletion of sEH (sEH $^{-/-}$) is associated with increased EET levels, increased $A_{2A}AR$ expression, and enhanced CRH. Mice which are $A_{2A}AR$–deficient ($A_{2A}AR^{-/-}$) had decreased CRH and increased expression of she, which breaks down the protective EETs to DHETs. It was shown previously that EETs mediated $A_{2A}AR$–induced vasodilation. Also, change in sEH activity is associated with corresponding changes in arachidonic and linoleic acids-derived oxylipin profiles, which have been demonstrated to have vasoactive effects. One important group of the evaluated oxylipins is HETEs; both mid-chain and ω-terminal HETEs have vasoconstrictive effects and decrease CRH. In isolated mouse hearts, the relationship among $A_{2A}AR$–deletion, subsequent oxylipin changes, sEH, ω-hydroxylases, and modulation of CRH is not known. Therefore, this study was designed to investigate the changes in oxylipin profiles associated with $A_{2A}AR$–deficiency ($A_{2A}AR^{-/-}$ mice) and the impact of sEH and ω -hydroxylases on the decreased CRH in the absence $A_{2A}AR$ in mice. Our data demonstrated that: **1)** Blocking $A_{2A}AR$ (by SCH 58261) decreased CRH in both wild type and sEH $^{-/-}$ mice; **2)** Inhibition of sEH (by *t*-AUCB)

reversed the decrease in CRH associated with A_{2A}AR–deletion in $A_{2A}AR^{-/-}$ mice; **3)** Inhibition of ω-hydroxylases (by DDMS) reversed the decrease in CRH associated with A_{2A}AR–deletion in $A_{2A}AR^{-/-}$ mice; **4)** A_{2A}AR–deletion was associated with changes in plasma oxylipin profiles, including decrease in EET/DHET ratios, increase in mid-chain and ω-terminal HETEs, decrease in EpOME/DiHOME ratios, increase in 9-HODE, and increase in prostanoids; **5)** A_{2A}AR–deletion was also associated with changes in oxylipin profiles in heart perfusate, with increase in DHET, and increase in mid-chain HETEs.

A_{2A}AR–antagonist (SCH 58261) intensely decreased CRH in both wild type and sEH $^{-/-}$ mice. A_{2A}AR mediated CRH in mice [40] and dogs [8]. SCH 58261 reduced CRH in response to 15-second ischemia in dogs [8]. Still, the effect of SCH 58261 on CRH in $sEH^{-/-}$ mice has not been reported. CRH is the cardiac response to brief ischemia [2]; its goal is to restore cardiac perfusion [1], and thus may protect the heart against ischemia's potential damage [9]. The importance of CRH is reiterated by several investigations which linked reduced CRH to cardiovascular pathologies affecting the coronary arteries [4-6]. We aimed to investigate the interaction between A_{2A}AR and sEH in regards to CRH. Adenosine's effects are mediated by four distinct receptor subtypes: A_1, A_{2A}, A_{2B} and A_3 [33]. Both A_{2A}AR and A_{2B}AR mediate the cardioprotective effects of adenosine [27]. The relationship between adenosine A_{2A}AR and CYP-epoxygenase–EET pathway is multifaceted. A_{2A}AR–activation resulted in increased EETs' release [32], and $A_{2A}AR^{-/-}$ (A_{2A}-null) mice had increased expression of EETs'–metabolizing enzyme sEH [33]. Additionally, our lab has reported that $sEH^{-/-}$ mice had increased aortic expression of A_{2A}AR [41] and increased EET levels [10]. At the functional level, $sEH^{-/-}$ mice demonstrated more aortic relaxation in response to an A_{2A}AR–agonist, which is consistent with the increased expression of A_{2A}AR [41], and enhanced CRH, which is in line with the increase in EET levels [10]. In this study, blocking A_{2A}AR attenuated CRH in $sEH^{-/-}$ mice such that it became comparable to that of wild type. In other words, the molecular changes associated with sEH–deletion (increased EET levels and increased expression of A_{2A}AR), which promote vasorelaxation, were defused by blocking A_{2A}AR. Therefore, these data suggest that blocking A_{2A}AR attenuates CRH partly through inhibiting A_{2A}AR–induced increase in EETs' release; an observation that supports previous findings of a link between A_{2A}AR activation and EETs' release [32, 33].

In $A_{2A}AR^{-/-}$ mice, CRH was decreased in response to brief ischemia compared to WT mice. As mentioned earlier, A_{2A}AR–activation resulted in increased EETs' release [32], and $A_{2A}AR^{-/-}$ mice had increased expression of EETs'–metabolizing enzyme sEH [33]. EETs are hydrated to their corresponding metabolites, DHETs, by sEH [42]. Increased EETs or EET/DHET ratios partly mediate the enhanced CRH in sEH–deleted [10] and in sEH–inhibited mouse heart in response to brief ischemia [9]. At the same time, endothelial over-expression of human sEH decreased CRH in isolated mouse heart [3], increased conversion of EETs to DHETs, and impaired endothelium-dependent cerebral vasodilation in mice [18]. These and other reports reconfirm the beneficial cardiovascular effects of EETs [25, 42-44], including vasodilation in renal preglomerular vasculature [45], intestinal vasculature [46], and brain vessels [47], and protection against ischemia/reperfusion injury [15]. The A_{2A}AR–EET pathway was demonstrated by several studies; EETs mediated A_{2A}AR–induced

vasodilation in rat arcuate arteries [31]. Also, $A_{2A}AR$–activation was followed by increased EETs' release in isolated perfused kidney [32]. Our lab revealed yet another facet of the interaction between CYP-epoxygenase–EET pathway and adenosine $A_{2A}AR$, where $A_{2A}AR^{-/-}$ (A_{2A}-null) mice had increased sEH expression compared to $A_{2A}AR^{+/+}$ (wild type) mice [33]. The increased sEH expression is a common finding between this mouse genotype, $A_{2A}AR^{-/-}$, and Tie2-sEH Tr mice, which had endothelial-specific over-expression of sEH [3]; they both had decreased CRH compared to their controls [3]. The increased expression of sEH was also associated with increased conversion of EETs to DHETs [18], which would decrease EET/DHET ratios. In this study, pharmacologic inhibition of sEH, by *t*-AUCB, reversed the decreased CRH observed in $A_{2A}AR^{-/-}$ mice and decreased EET/DHET ratios (as mentioned below). sEH role in CRH has been repeatedly demonstrated [3, 9, 10]. Therefore, the ability of an sEH–inhibitor to reverse the decreased CRH in $A_{2A}AR^{-/-}$ mice, suggests that the absence of A_{2A} receptors in $A_{2A}AR^{-/-}$ mice could be associated with increased activity of sEH; a possibility which was supported by the measured EETs and DHETs in heart perfusate and plasma as demonstrated below.

Oxylipins were analyzed in plasma and heart perfusate of $A_{2A}AR^{-/-}$ and $A_{2A}AR^{+/+}$ mice. The heart perfusate data have the advantage of demonstrating the impact of ischemia on oxylipin levels as they are assessed before and after the ischemic interruption of perfusion. However, collecting the heart perfusate poses a number of challenges including subjecting the functioning heart to the air while moving it from its warm water-jacketed buffer bath to the collection beaker, which could affect its overall functions. Besides, the collection beaker has to contain warmed buffer to simulate the warm bath it is usually placed in before collection; this entails that the collected perfusate is further diluted, and probably partially accounts for the high variability we noticed in oxylipin levels in previous experiments [3, 9-11]. Additionally, the diluted heart perfusate samples lower the available oxylipins to undetectable ranges as noticed for some of the oxylipins we tried to measure in this study. Therefore, we sought to measure the oxylipin levels in the plasma. This approach allowed us to have more accurate readings of oxylipins with less variability, detected oxylipins which were undetectable in the heart perfusate, and offered a unique chance to compare the oxylipin data between the two sources: plasma and heart perfusate. In the current study, deletion of $A_{2A}AR$ ($A_{2A}AR^{-/-}$ mice) was associated with increased levels of DHETs compared to WT mice in the heart perfusate. However, we could not detect EETs in the same samples. We experienced similar difficulty in detecting all EETs in the heart perfusate samples in previously published work [9] and this could be attributed to the short half-life of EETs [48]. DHETs are the sEH–catalyzed metabolic breakdown products of EETs. The plasma levels of both EETs and DHETs supported the findings in the heart perfusate samples; EET/DHET ratio was decreased by the elevated DHET levels. We earlier reported that global deletion ($sEH^{-/-}$ mice) [9] and pharmacologic inhibition of sEH [9] decreased DHETs in heart perfusate. The increased expression of sEH reported in $A_{2A}AR^{-/-}$ mice [33] is probably what caused the observed increase in DHETs' generation in our study and drove the decrease in EET/DHET ratios in this mouse genotype. EETs have anti-inflammatory properties [49]. In mouse carotid arteries, EETs prevented leukocyte adhesion and decreased the number of adherent mononuclear cells to the vascular wall by inhibiting the transcription of NF-κB following TNF-α injection; an independent effect of their membrane-

hyperpolarizing effects [50]. This observation in $A_{2A}AR^{-/-}$ mice, along with the other plasma oxylipin data below, is interesting since it suggests a possible decrease in the anti-inflammatory role of EETs. Therefore, the attenuated CRH in $A_{2A}AR^{-/-}$ mice could be partially due to decreased EET/DHET ratio and increased DHETs, which could be attributed to the increased expression of EETs' metabolizing enzyme (sEH) and may indicate an overall reduction in EETs vasodilatory and anti-inflammatory activity in $A_{2A}AR^{-/-}$ mice.

Mid-chain (5-, 8-, 11-, 12- and 15-) HETE levels were increased in $A_{2A}AR^{-/-}$ compared to WT mice. Lipoxygenase produces mid-chain HETEs by allylic oxidation from AA [24]. CYP-epoxygenase 1B1 also produces mid-chain HETEs by bis-allylic oxidation [51]. Mid-chain HETEs have pro-inflammatory and vasoconstrictive effects [51, 52]. Also, cardiovascular dysfunction was associated with increased formation of mid-chain HETEs [53-56]. Both of our samples, heart perfusate and plasma, demonstrated increase in mid-chain HETEs in $A_{2A}AR^{-/-}$ compared to WT mice. Based on that, the increase in mid-chain HETEs in $A_{2A}AR^{-/-}$ mice, along with the decreased EET/DHET ratios, indicates an increase in the proinflammatory state associated with $A_{2A}AR$–deletion, and may have contributed to the decreased CRH in $A_{2A}AR^{-/-}$ mice.

Like mid-chain HETEs, ω-terminal HETEs are generated from AA, but through CYP ω-hydroxylases, primarily CYP4A and CYP4F subfamilies [19]. The primary metabolite of ω-terminal HETEs is 20-HETE [19], which promotes hypertension, vasoconstriction, and vascular dysfunction [20, 21]. Waldman et al. demonstrated that blocking 20-HETE synthesis reduced mean arterial pressure in old spontaneously hypertensive female rats [57]. We have recently reported that inhibiting ω-hydroxylases by DDMS enhanced CRH in sEH-overexpressed (Tie2-sEH Tr) and control mice. Similarly, targeting the ω-terminal-HETEs pathway in this study reversed the decreased CRH in $A_{2A}AR^{-/-}$ mice. Interestingly, our lab reported that higher levels of the 20-HETE-generating enzyme, CYP4A, were expressed in the aorta of $A_{2A}AR^{-/-}$ compared to $A_{2A}AR^{+/+}$ mice [58]. Therefore, ω-hydroxylases–inhibition reversed the decreased CRH in $A_{2A}AR^{-/-}$ mice, which is consistent with the reported increased expression of CYP4A in the same mice [58]. We then went further to investigate the impact of CYP4A's increased expression on the levels of ω-terminal HETEs (19- and 20-HETEs), but they were too low to be detected in the heart perfusate by our technique, which we encountered and reported earlier [3]. Fortunately, we were able to detect these ω-terminal HETEs in plasma samples, and observed increased levels of ω-terminal HETEs, particularly 20-HETE. This observation may account for ω-hydroxylase–inhibitor's efficacy to reverse the decreased CRH in $A_{2A}AR$–deficient mice. Therefore, the increased 20-HETE in $A_{2A}AR^{-/-}$ mice, along with the other changes in oxylipins, suggests an increase in the proinflammatory state associated with $A_{2A}AR$–deficiency and may have contributed to the attenuated CRH in $A_{2A}AR^{-/-}$ mice.

Deletion of $A_{2A}AR$ in $A_{2A}AR^{-/-}$ mice was associated with increased plasma prostanoid levels, including PG-$F_{2\alpha}$, PG-E_2, PG-D_2, and T×B_2. Prostanoids include two groups of metabolites: thromboxanes (T×A_2 and T×B_2) and the four main bioactive prostaglandins (PG-$F_{2\alpha}$, PG-E_2, PG-I_2, PG-D_2) [59, 60]. Prostaglandins are generally pro-inflammatory [60]. For instance, microvascular permeability was increased by PG-E_2 [60]. 6-keto-PG-$F_{1\alpha}$, the inactive metabolite of prostacyclin (PG-I2), was not different between $A_{2A}AR^{-/-}$ and

WT mice [60]. T×B_2 is the inactive degradation product of T×A_2, which mediates platelet aggregation, smooth muscle contraction, and endothelial inflammation [60]. Since sEH-expression was higher in $A_{2A}AR^{-/-}$ mice [58], the observed increase in prostanoids is in agreement with our, as well as others', reported data that disrupting sEH activity, through pharmacologic inhibition or genetic deletion, was associated with decreased prostanoids [9, 61, 62]. Still, neither could we infer a relationship between prostanoid levels and CRH [3, 9, 10] nor did Hellmann et al., who suggested that PGs were not involved in post-occlusive reactive hyperemia in the skin [63]. Therefore, the increased prostanoid levels in $A_{2A}AR^{-/-}$ mice may have contributed to an increase in the proinflammatory state in $A_{2A}AR^{-/-}$ mice, but its impact on the attenuated CRH is yet to be determined.

The role of sEH in cardiovascular biology extends beyond its role in EET hydrolysis; it plays a central role in the metabolism of other arachidonic-, linoleic- and omega-3-derived oxylipins [24]. For example, EpOMEs, whose parental fatty acid is linoleic acid, are hydrolyzed to DiHOMEs by sEH. We reported earlier that global deletion of sEH ($sEH^{-/-}$ mice) [9] and pharmacologic inhibition of sEH by *t*-AUCB [9] increased EpOMEs, decreased DiHOMEs, and increased EpOME/DiHOME ratio in heart perfusate. In these two published studies, the increased EpOME/DiHOME ratio was believed to contribute to the enhancement of CRH [9], which suggested that increased EpOME/DiHOME ratio may have had a positive role in mediating vasodilation. A notion supported by other reports demonstrating a protective effect against hypoxia/reoxygenation injury by EpOMEs in primary cultures of rabbit renal proximal tubular cells; an effect which was unattainable with DiHOMEs [23]. On the other hand, endothelium- dependent vasodilation in the cerebral circulation was impaired by decreased EpOME/DiHOME ratio in Tie2-sEH Tr mice [18]. Likewise, in this study, $A_{2A}AR^{-/-}$ mice had decreased EpOME/DiHOME ratio driven by increased DiHOMEs in the plasma. DiHOMEs were reported to have deleterious properties, including cytotoxic, cardiodepressive and vasoconstrictive [17, 25]. Therefore, the decreased EpOME/DiHOME ratio and increased DiHOMEs in $A_{2A}AR^{-/-}$ mice may have contributed to the attenuated CRH in $A_{2A}AR^{-/-}$ mice.

The other oxylipins derived from linoleic acid (LA), in addition to EpOMEs, are HODEs, which are generated through hydroxylation of LA by CYP epoxygenases or lipoxygenases [24]. 9-HODE, but not 13-HODE, was increased in $A_{2A}AR^{-/-}$ compared to $A_{2A}AR^{+/+}$ mice. Although the physiologic functions of HODEs are not widely investigated [24], 9-HODE is thought to be pro-inflammatory [64, 65], whereas13-HODE could be anti-inflammatory [66-70]. The opposite effects of these two isomers makes this observation consistent with earlier reported finding by our lab of increased 13–HODE level in sEH–deleted ($sEH^{-/-}$) mice [9]. The increased 9-HODE in $A_{2A}AR^{-/-}$ mice may be linked to the reported increase in sEH-expression in $A_{2A}AR^{-/-}$ mice [33]. Thus, the increased 9-HODE in $A_{2A}AR^{-/-}$ mice possibly have contributed to the attenuated CRH in $A_{2A}AR^{-/-}$ mice.

Conclusion

The findings of this study suggest that the previously established link between $A_{2A}AR$–activation and EETs' release in the aortic tissue is also valid in the coronary arteries. Also, our data demonstrated that the decreased CRH associated with $A_{2A}AR$–deficiency can be

reversed by augmenting the CYP-epoxygenase–EETs pathway (through sEH–inhibition) and by blocking the ω-hydroxylase pathway (through CYP4A–inhibition). To the best of our knowledge, this is the first time oxylipins are evaluated in $A_{2A}AR^{-/-}$ mice from the perspective of coronary reactive hyperemia. The changes in heart perfusate and plasma oxylipins were consistent and may partly be responsible for the reduced CRH, including decreased EET/DHET ratio, increased mid-chain and ω-terminal HETEs, decreased EpOME/DiHOME ratio, and increased 9-HODE. Also, these changes in oxylipins, along with the increase in prostanoids, suggest an increased proinflammatory state in $A_{2A}AR^{-/-}$ mice.

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Highlights

- Adenosine $A_{2A}AR$ is involved in the cardiprotective coronary reactive hyperemia (CRH) in response to ischemia.
- Soluble epoxide hydrolase (sEH) breaks down epoxyeicosatrienoic acids (EETs), which are cardioprotective metabolites.
- $A_{2A}AR$ deletion is associated with changed oxylipin profiles, which were consistent between plasma and heart perfusate samples, and indicate an increased proinflammatory state including increased ω-terminal HETEs, decreased epoxyoctadecaenoic / dihydroxyoctadecaenoic acids ratios, increased 9-hydroxyoctadecadienoic acid, and increased prostanoids.
- Inhibition of either sEH or ω-hydroxylases reversed the reduced CRH in $A_{2A}AR^{-/-}$ mice.

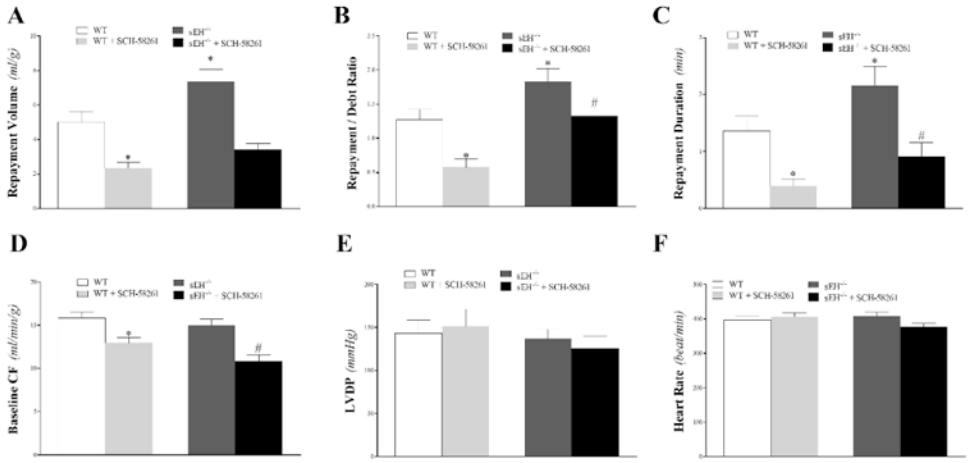

Fig 1. Effect of the selective A_{2A}AR–antagonist (SCH-58261, 0.1 μM) on coronary reactive hyperemia (CRH) in WT and sEH$^{-/-}$ mice

The selective A_{2A}AR–antagonist, SCH-58261, decreased CRH in both WT and sEH$^{-/-}$ mice. Repayment volume (A), repayment/debt ratio (B), and repayment duration (C) were more increased in sEH$^{-/-}$ compared to WT mice. They, and baseline CF (D), were decreased by SCH-58261 in both WT and sEH$^{-/-}$ mice. No significant difference between SCH-58261–treated WT and SCH-58261–treated sEH$^{-/-}$ mice in the above–mentioned parameters was observed. Baseline CF (D), LVPD (E), and HR (F) were not different between and within the two groups. * $P < 0.05$ versus untreated WT. # $P < 0.05$ versus SCH-58261–treated WT. $n = 8$ per group.

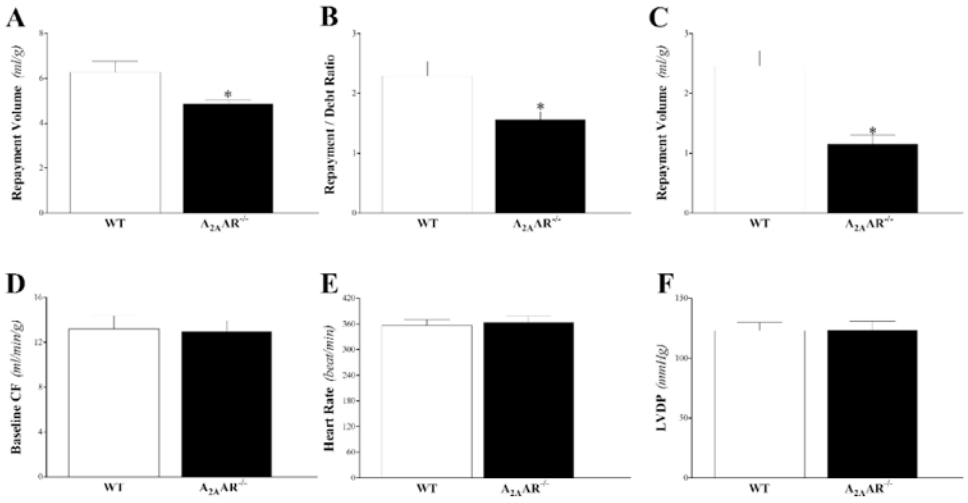

Fig 2. Comparison of coronary reactive hyperemia (CRH) between WT and $A_{2A}AR^{-/-}$ mice

Repayment volume (A), repayment/debt ratio (B), and repayment duration (C), were decreased in $A_{2A}AR^{-/-}$ compared to WT mice ($P < 0.05$). Baseline CF (D), LVPD (E), and HR (F) were not different between the two groups. * $P < 0.05$ versus $A_{2A}AR^{-/-}$. $n = 8$ per group.

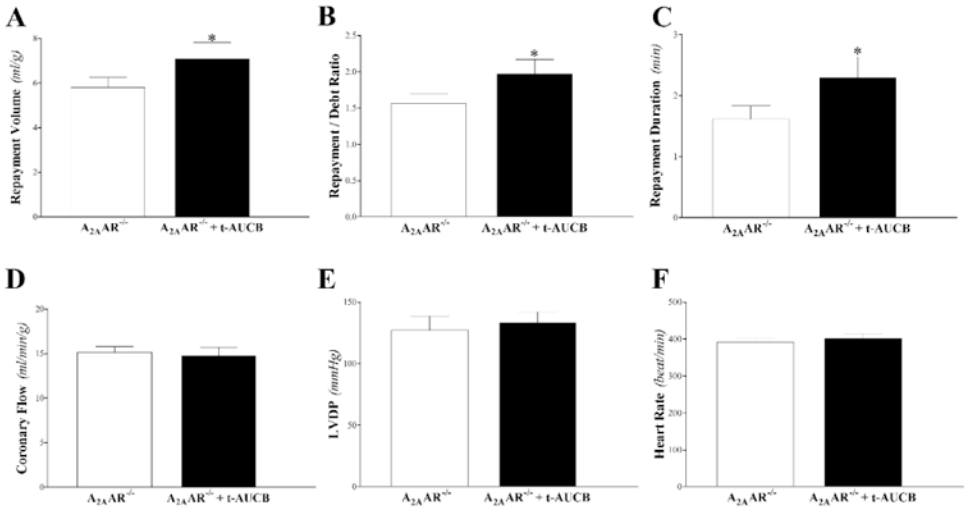

Fig 3. Effect of the selective sEH-inhibitor (*t*-AUCB, 10 μM) on coronary reactive hyperemia (CRH) in $A_{2A}AR^{-/-}$ mice

Repayment volume (A), repayment/debt ratio (B), and repayment duration (C), were enhanced in $A_{2A}AR^{-/-}$ mice by *t*-AUCB ($P < 0.05$). Baseline CF (D), LVPD (E), and HR (F) were not different between the two groups. * $P < 0.05$ versus $A_{2A}AR^{-/-}$. $n = 10$ per group.

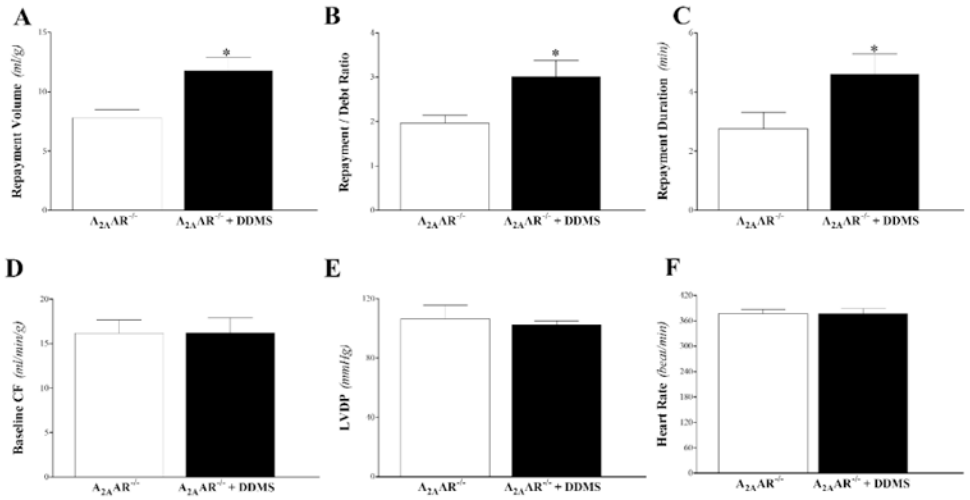

Fig 4. Effect of the ω-hydroxylases-inhibitor (DDMS, 1 μM) on coronary reactive hyperemia (CRH) in $A_{2A}AR^{-/-}$ mice

Repayment volume (A), repayment/debt ratio (B), and repayment duration (C), were enhanced in $A_{2A}AR^{-/-}$ mice by DDMS ($P < 0.05$). Baseline CF (D), LVPD (E), and HR (F) were not different between the two groups. * $P < 0.05$ versus $A_{2A}AR^{-/-}$. $n = 6$ per group.

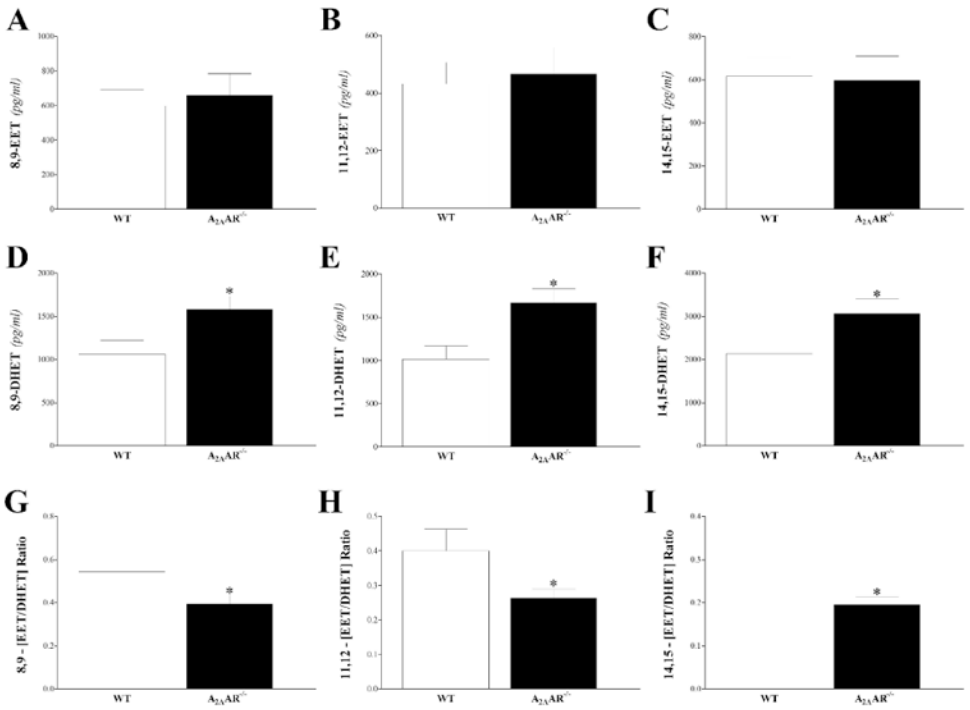

Fig 5. LC–MS/MS analysis for DHETs (11, 12–, and 14, 15–DHETs) levels in WT and $A_{2A}AR^{-/-}$ mouse heart perfusate at baseline and post-ischemia

Both 11,12-, and 14,15-DHETs were increased in $A_{2A}AR^{-/-}$ vs. WT mice at baseline and post-ischemia. Only 11,12-DHET's increase (A) was statistically significant. * $P < 0.05$ versus WT. $n = 6$ per group.

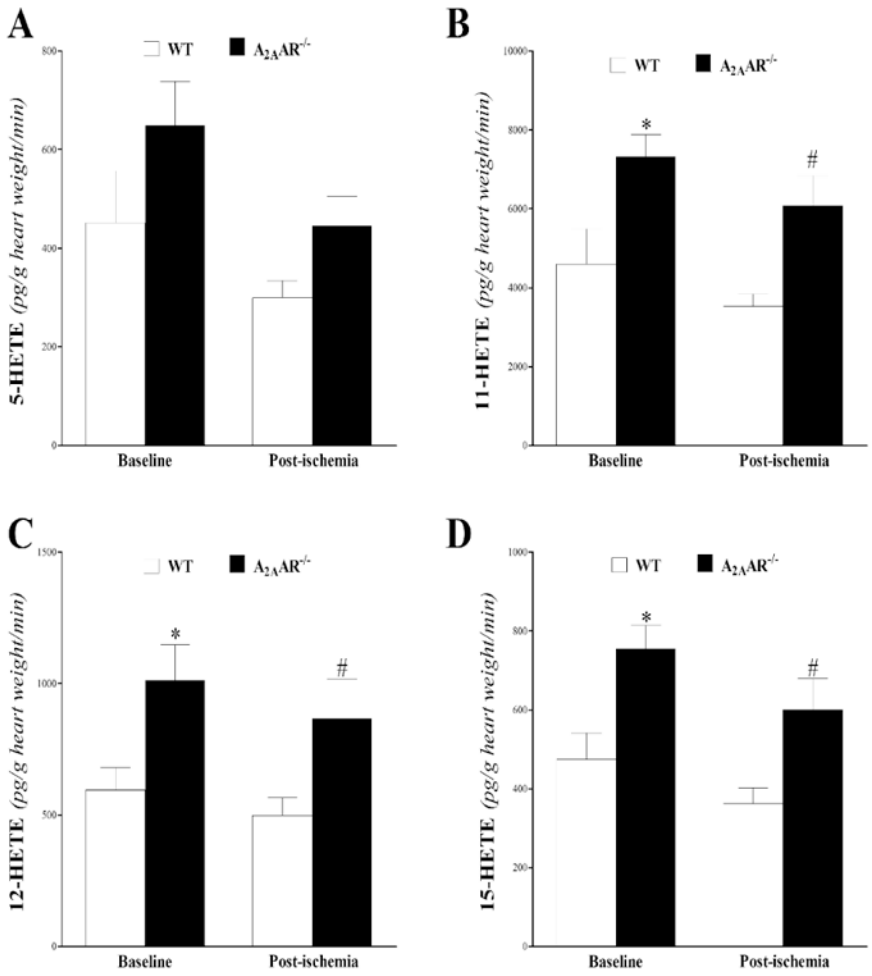

Fig 6. LC–MS/MS analysis for EETs (8, 9–, 11, 12–, and 14, 15–) and DHETs (8, 9–, 11, 12–, and 14, 15–) levels in WT and $A_{2A}AR^{-/-}$ mouse plasma

Plasma 8,9-EET (A), 11,12-EET (B), and 14,15-EET (C) were not different, whereas, 8,9-DHET (D), 11,12- DHET (E), and 14,15- DHET (F) were increased in $A_{2A}AR^{-/-}$ compared to WT mice. As a result, 8,9- (G), 11,12- (H), and 14,15- (I) EET/DHET ratios decreased in $A_{2A}AR^{-/-}$ compared to WT mice. * $P < 0.05$ versus WT. $n = 10$ per group.

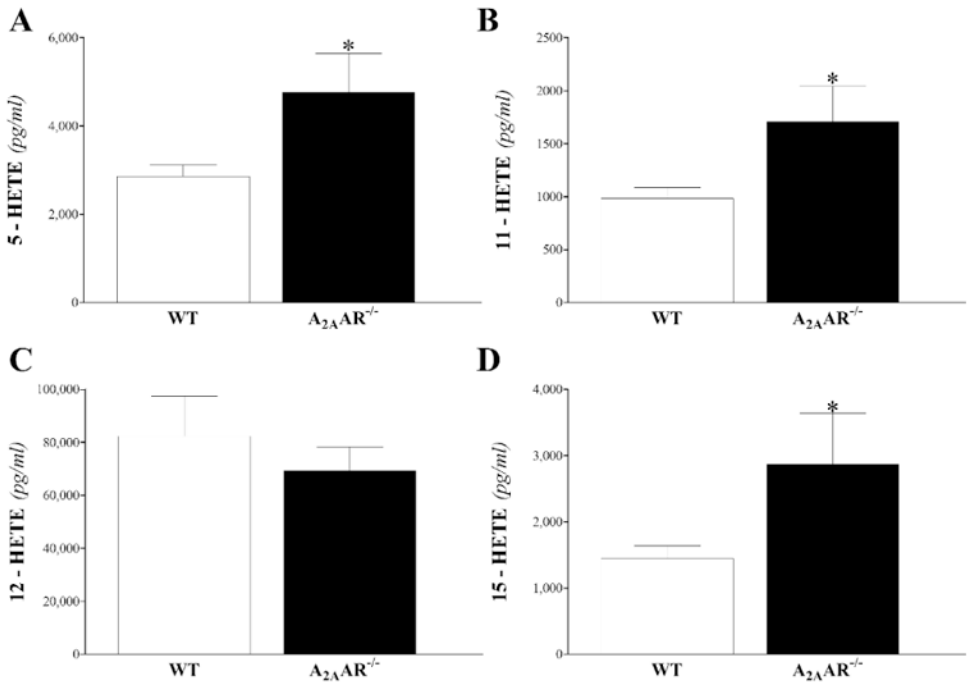

Fig 7. LC–MS/MS analysis for mid-chain HETE (5-, 11-, 12-, and 15-HETE) levels in WT and $A_{2A}AR^{-/-}$ mouse heart perfusate at baseline and post-ischemia

Baseline and post-ischemic levels of 5- (A), 11- (B), 12- (C), and 15- (D) HETE were increased in $A_{2A}AR^{-/-}$ compared to WT mice, and was significant for 11-, 12-, and 15-HETEs. * $P < 0.05$ versus WT. $n = 6$ per group.

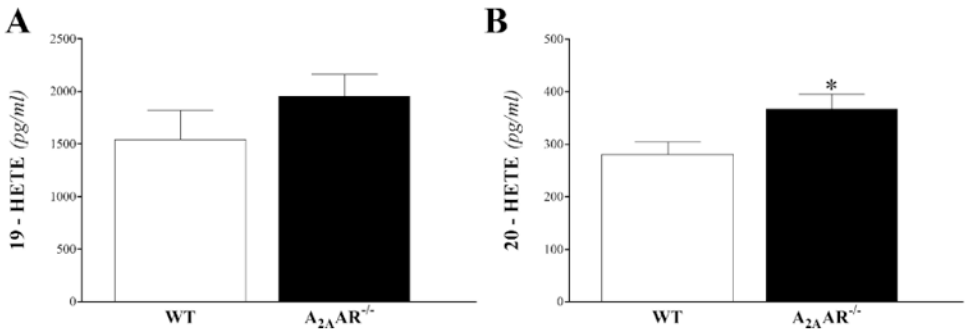

Fig 8. LC–MS/MS analysis for mid-chain HETE (5-, 11-, 12-, and 15-HETE) levels in WT and $A_{2A}AR-/-$ mouse plasma

Plasma 5- (A), 11- (B), and 15- (D) HETEs, but not 12- (C) HETE, were significantly increased in $A_{2A}AR^{-/-}$ compared to WT mice. * $P < 0.05$ versus WT. $n = 9$ per group.

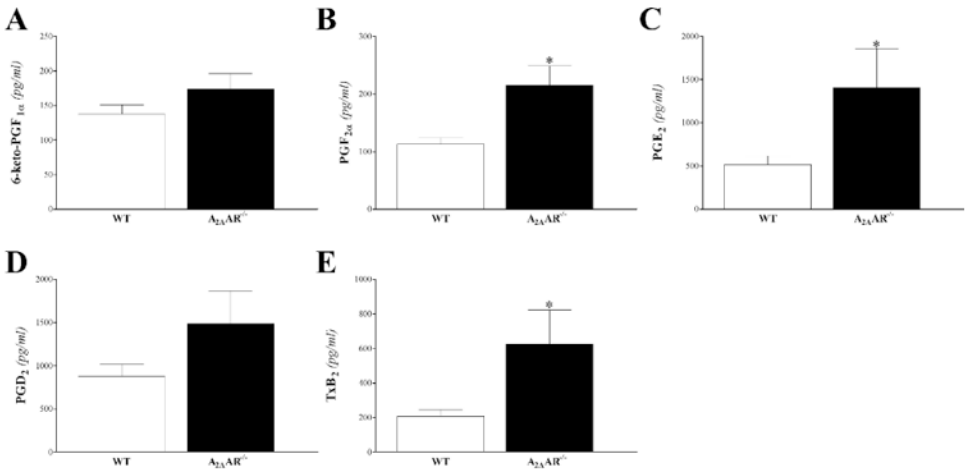

Fig 9. LC–MS/MS analysis for ω–Terminal HETEs (19- and 20-HETE) levels in WT and $A_{2A}AR^{-/-}$ mouse plasma

Plasma 19-HETE (A) and 20-HETE (B) were increased in $A_{2A}AR^{-/-}$ compared to WT mice, but only 20-HETE was statistically significant. * $P < 0.05$ versus WT. $n = 9$ per group.

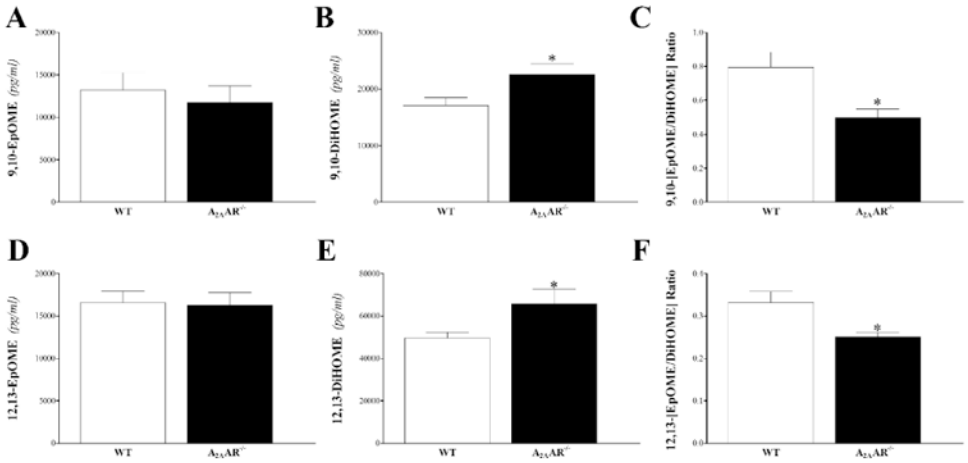

Fig 10. LC-MS/MS analysis for prostanoids (6-keto-PG-$F_{1\alpha}$, PG-$F_{2\alpha}$, PG-D_2, PG-E_2, and T×B_2) levels in WT and $A_{2A}AR^{-/-}$ mouse plasma

Plasma 6-keto-PG-$F_{1\alpha}$ (A), PG-$F_{2\alpha}$ (B), PG-E_2 (C), PG-D_2 (D), and T×B_2 (E) were increased in $A_{2A}AR^{-/-}$ compared to WT mice, but were significant for PG-$F_{2\alpha}$ (A), PG-E_2 (C), and T×B_2 (E). * $P < 0.05$ versus WT. $n = 9$ per group.

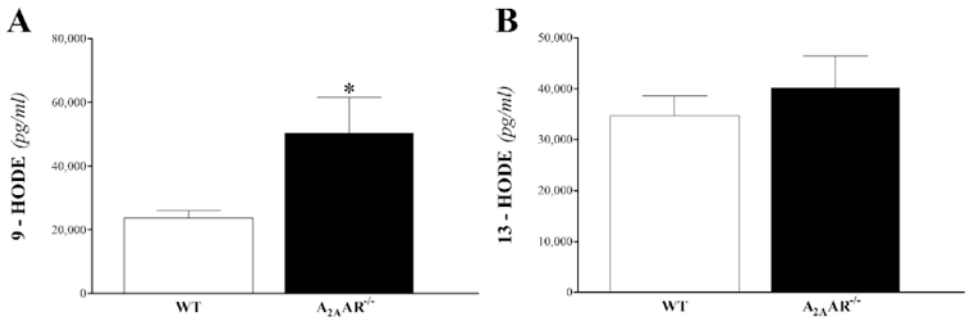

Fig 11. LC–MS/MS analysis for EpOME and DiHOME levels in WT and $A_{2A}AR^{-/-}$ mouse plasma

Compared to WT, $A_{2A}AR^{-/-}$ mice had similar levels of plasma 9,10- (A) and 12,13-EpOMEs (D), increased 9,10- (B) and 12,13-DiHOMEs (E), and as a result, decreased 9,10- (C) and 12,13- EpOME/DiHOME ratios (F). * $P < 0.05$ versus WT. $n = 9$ per group.

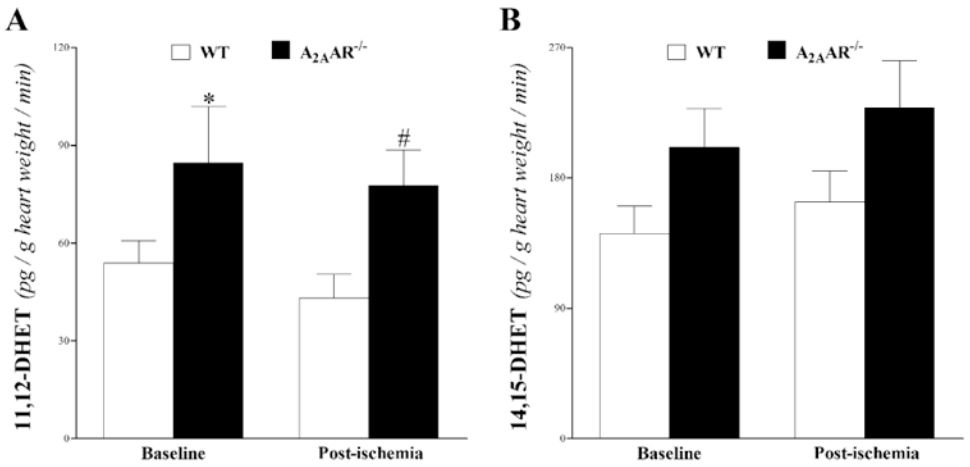

Fig 12. LC–MS/MS analysis for HODE levels in WT and $A_{2A}AR^{-/-}$ mouse plasma

Plasma 9-HODE (A), but not 13-HODE (B), was increased in $A_{2A}AR^{-/-}$ compared to WT. * $P < 0.05$ versus WT. $n = 9$ per group.

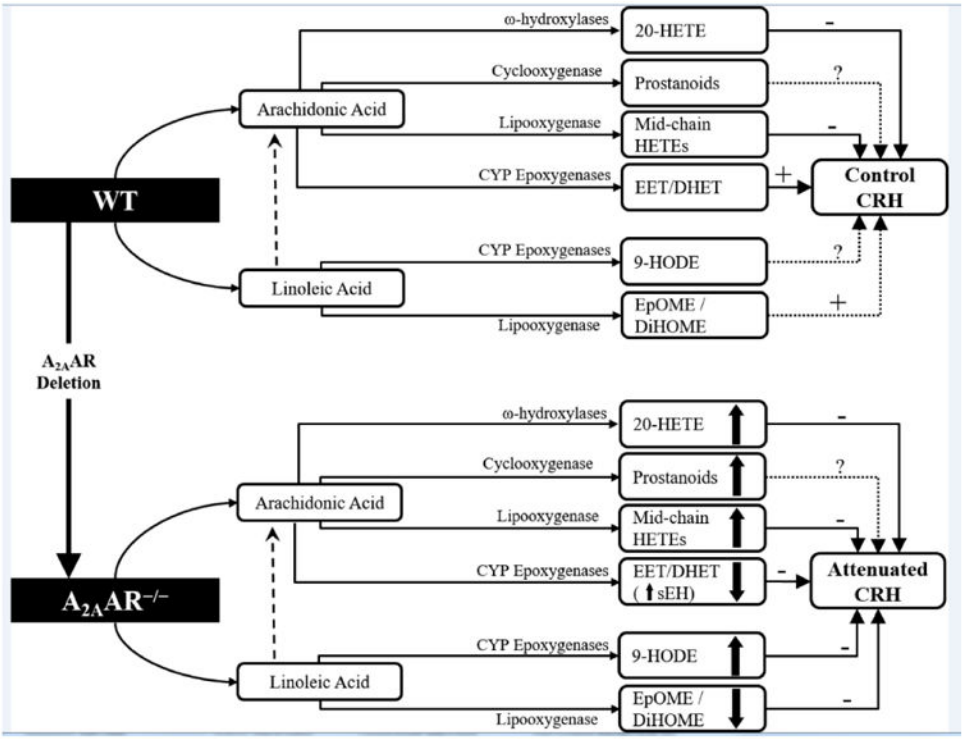

Fig 13. A schematic diagram comparing the changes in heart perfusate and plasma oxylipin profiles observed in response to $A_{2A}AR$-deletion as well as their possible effect on coronary reactive hyperemia (CRH) in WT and $A_{2A}AR^{-/-}$ mice

The observed changes in the measured heart perfusate and plasma oxylipin profiles collectively resulted in attenuated CRH in $A_{2A}AR^{-/-}$ compared to WT mice. These changes included increased plasma 20-HETE, increased heart perfusate and plasma mid-chain HETEs, decreased heart perfusate and plasma EET/DHET ratio, increased plasma 9-HODE, and decreased plasma EpOME/DiHOME ratio. It is not clear what impact the increased prostanoid levels has on CRH, but it may, along with the other changes in oxylipins, contribute to the increased proinflammatory state in $A_{2A}AR^{-/-}$ mice.

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Table 1

Oxylipin concentration in plasma samples (pg / mL) determined by LC-MS/MS in wild type (WT) and $A_{2A}AR$-null ($A_{2A}AR^{-/-}$) mice.

Oxylipin	WT			$A_{2A}AR^{-/-}$		
Arachidonic Acid-derived Oxylipins						
8,9-EET (pg/mL)	598	±	94	660	±	125
11,12-EET (pg/mL)	432	±	76	467	±	91
14,15-EET (pg/mL)	615	±	88	598	±	110
8,9-DHET (pg/mL)	1,062	±	162	1,585	±	150 *
11,12-DHET (pg/mL)	1,014	±	155	1,666	±	165 *
14,15-DHET (pg/mL)	2,136	±	306	3,062	±	343 *
8,9- (EET/DHET) ratio	0.56	±	0.05	0.38	±	0.03 *
11,12- (EET/DHET) ratio	0.40	±	0.05	0.32	±	0.05 *
14,15- (EET/DHET) ratio	0.30	±	0.03	0.27	±	0.05 *
5-HETE (pg/mL)	2,854	±	262	4,772	±	878 *
11-HETE (pg/mL)	981	±	106	1,710	±	334 *
12-HETE (pg/mL)	82,335	±	15,143	69,265	±	8,986
15-HETE (pg/mL)	1,441	±	197	2,870	±	772 *
19-HETE (pg/mL)	1,542	±	277	1,958	±	204
20-HETE (pg/mL)	281	±	23	367	±	28 *
6-keto-$PGF_{1\alpha}$ (pg/ml)	138	±	13	174	±	22
$PGF_{2\alpha}$ (pg/ml)	113	±	11	215	±	34 *
PGD_2 (pg/ml)	879	±	139	1,488	±	376
PGE_2 (pg/ml)	514	±	105	1,410	±	451 *
$T\times B_2$ (pg/ml)	209	±	38	624	±	198 *
Linoleic Acid-derived Oxylipins						
9,10-EpOME (pg/mL)	13,198	±	2,068	11,782	±	1,913
12,13-EpOME (pg/mL)	16,553	±	1,375	16,270	±	1,482

Oxylipin	WT			$A_{2A}AR^{-/-}$		
9,10-DiHOME (pg/mL)	17,089	±	1,340	22,560	±	1,939 *
12,13-DiHOME (pg/mL)	49,477	±	2,978	65,670	±	6,909 *
9,10-(EpOME/DiHOME) ratio	0.80	±	0.09	0.50	±	0.05 *
12,13-(EpOME/DiHOME) ratio	0.33	±	0.03	0.25	±	0.01 *
9-HODE (pg/mL)	23,725	±	2,327	50,297	±	11279 *
13-HODE (pg/mL)	34,629	±	3,942	40,159	±	6,274

* $P < 0.05$ versus WT. Values are means ± standard error. $n = 10$ per group.