

Arachidonic Acid and GPR39 Modulate Coronary Autoregulation

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Abstract

The molecular basis of in vivo myogenic response and coronary autoregulation is unknown. We hypothesized that arachidonic acid (AA) participates in autoregulation through its metabolite, 15-hydroxyeicosatetraenoic acid (15-HETE), which activates GPR39 present in coronary arteriolar vascular smooth muscle cells (VSMCs). 15-HETE is the endogenous agonist for GPR39 and causes VSMC contraction by increasing cytosolic Ca^{++} through $\text{G}\alpha_q$ activation. Accordingly, isolated hearts from 103 wild type (WT) and 8 GPR39 knock out (KO) mice were perfused in a Langendorff system where coronary driving pressure (CDP) could be altered over a wide range ($< 40\text{--}150$ mmHg). WT hearts were perfused with modified Krebs-Henseleit buffer (mKHB) solution ($n = 9$), 10% plasma+mKHB ($n = 7$), and 3% fatty acid free albumin (FAFA)+mKHB ($n = 8$) over a wide range of CDPs. Autoregulation was only seen with plasma+mKHB. Since AA is the most abundant albumin-bound fatty acid in plasma, we infused different doses of AA with FAFA+mKHB ($n = 36$) at various CDPs in WT mouse hearts. Autoregulation was noted when 16 nmol AA was infused over 5 min and not at lower doses. The combination of mKHB+FAFA + AA produced autoregulation in all 10 WT hearts but not in any of the 8 GPR39 KO hearts. Because AA is released from plasma membrane by phospholipase A_2 (PLA_2), adding a specific PLA_2 inhibitor (palmitoyl trifluoromethyl ketone, $10\mu\text{M}$ and $50\mu\text{M}$) abolished autoregulation ($n = 12$) compared to vehicle ($n = 7$). A nonspecific Ca^{++} blocker, as well as inositol 1,4,5-trisphosphate, and transient receptor potential channel inhibitor (2-aminoethoxydiphenyl borate) almost abolished autoregulation in the doses used ($n = 15$) compared to vehicle ($n = 7$). We conclude that AA and GPR39 participate in the in vivo myogenic response that forms the basis of coronary autoregulation. In addition, PLA_2 and mechanotransduction of coronary intraluminal pressure complete the feedback loop required for autoregulation.

Introduction

Coronary autoregulation is the ability of the heart to maintain constant coronary blood flow (CBF) over a wide range of coronary driving pressures (CDPs) so long as the oxygen requirements of the heart do not change [1–3]. Coronary autoregulation is accomplished by vasomotion of resistance arterioles ($50\text{ }\mu\text{m}$ to $250\text{ }\mu\text{m}$, depending on the species) that constrict when CDP rises and relax when it falls [3]. When vasomotion is exhausted at either end of the autoregulatory range, then CBF becomes CDP dependent [1,2].

Coronary vasomotion has been attributed to the myogenic response that is purported to be an inherent property of arteriolar vascular smooth muscle cells (VSMCs). VSMCs are thought to exhibit a reflex response to changes in intraluminal pressure (Bayliss effect). Myogenic response has been studied ex-vivo when an artery or a large arteriole has been isolated from the heart and suspended in an in vitro circulatory system, where arterial contractions can be measured in response to changes in pressure within the system [4]. Whether results of these in vitro studies are pertinent to the in vivo situation is unclear and the molecular mechanism of the in vivo myogenic response remains unknown.

There is ample evidence from ex-vivo beating heart preparations, that when perfused with crystalloid cardioplegia or Krebs buffer solutions, rodent hearts do not demonstrate either the myogenic response or autoregulation [5–9]. CBF in these hearts is entirely CDP dependent except when plasma or blood is added. Plasma has several bioactive molecules, including fatty acids of which arachidonic acid (AA) is the most abundant, with 99.9% bound to albumin. When untreated albumin, not stripped of bound molecules, is mixed with the perfusate, autoregulation is seen, implying that the moiety associated with autoregulation is albumin bound [6]. Conversely when charcoal treated albumin (where all fatty acids are removed) is infused autoregulation is not seen [6].

AA is converted into active metabolites via several enzymatic pathways. One conversion is to 15-Hydroxyeicosatetraenoic acid (15-HETE) by 15-lipoxygenase (15-LO) [10, 11]. Using a canine model of graded coronary stenoses, we found that 15-HETE levels decreased in step with reduced CDP when CBF was normal (autoregulatory range) [12]. In this setting we saw no change in the vasodilator 14,15-epoxyeicosatrienoic acid (14,15-EET). We also discovered that 15-HETE is the endogenous agonist for GPR39 in mouse cardiac arteriolar VSMCs where it increases cytosolic Ca^{++} that causes VSMC contraction [13]. 14,15-EET blocks this effect. GPR39 has basal constitutive activity [14] and Zn^{++} acts as a positive allosteric modulator for 15-HETE and 14,15-EET [15].

Given this background we hypothesized that the amplitude of mechanotransduction signal in the microcirculation is dependent on ambient arterial pressure. This signal stimulates phospholipase A_2 (PLA₂) in the plasma membrane, releasing AA that is then converted to 15-HETE by 15-LO. 15-HETE in turn activates GPR39 causing release of cytosolic Ca^{++} resulting in VSMC contraction. We postulated that when ambient coronary arteriolar pressure is high, this pathway is activated more than when the pressure is low, resulting in autoregulation. So, the range of CDPs during coronary autoregulation is directly regulated by fluctuations in 15-HETE levels.

Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Oregon Health & Science University (OHSU) and adhered to National Institutes of Health, USA for the Care and Use of Laboratory Animals. All reporting is based on ARRIVE guidelines. One-hundred and three male wild type (WT) C57BL/6 mice (10–16 weeks old) were obtained from Jackson Laboratory (Bar Harbor, ME, USA) and 8 similarly aged GPR39 knockout (KO) mice were obtained from Cyagen Biosciences (Santa Clara, CA, USA) [13]. All animals underwent the same surgical procedure.

Langendorff Isolated Perfused Mouse Heart Preparation

Mice received 100 IU of heparin intraperitoneally, 10 min prior to surgery. Anesthesia was induced using 3% inhaled isoflurane followed by euthanasia via cervical dislocation after all reflexes were abolished. The heart was rapidly exposed via a clamshell thoracotomy, excised, and transferred to a dish containing

ice-cold modified Krebs-Henseleit buffer (mKHB, pH 7.4; composition in mM: NaCl 118, NaHCO₃ 25, D-glucose 5.5, sodium pyruvate 2.0, KCl 4.7, MgSO₄ 1.6, KH₂PO₄ 1.2, and CaCl₂ 1.5).

After the aorta was transected between the first and second branches, it was cannulated using a shortened, blunt-ended 20-gauge needle and securely tied to the cannula groove with 5 – 0 silk suture. This needle has a side-arm for drug administration. The cannulated heart was then mounted on a Langendorff system (Hugo Sachs Elektronik Langendorff Perfusion System, Harvard Apparatus, Natick, MA, USA) and perfused retrogradely via the aorta with oxygenated mKHB maintained at 37°C using a heat exchanger (Fig. 1) [16]. The heart itself was suspended in a heating chamber whose temperature was maintained at 37°C. The temperature of the heat exchanger and heating chamber was maintained by a thermostat-controlled water bath.

The perfusate was continuously oxygenated with a gas mixture of 95% O₂ and 5% CO₂. CDP and CBF were continuously recorded using in-line pressure sensor (PM-4, Living Systems Instrumentation, St. Albans, VT, USA) and flow probe (TS410, Transonic Systems, Ithaca, NY), respectively (Fig. 1). Signals were digitized with a Digidata 1440A (Molecular Devices, Santa Jose, CA, USA) and analyzed using AxoScope 10.7 software (Molecular Devices).

The hearts were equilibrated at an initial pressure of 80 mmHg for at least 20 min before changing CDP, which was regulated by adjusting pressure in the arterial perfusate chamber. It was adjusted every 3 min in steps of approximately 10 mm Hg over a range of 30 to 140 mm Hg. At each step, CBF was recorded. Animals with the following attributes were not used for further experimentation: interval from euthanasia to cannulation > 4 min, coronary flow > 5 mL/min, bradycardia (heart rate < 250 beats per min), or tachycardia (heart rate > 600 beats per min).

Statistical analysis methods

Data in aggregate plots are presented as mean ± standard error of the mean (SEM). The primary outcome for statistical comparison was the slope of the coronary driving pressure (CDP) versus coronary blood flow (CBF) relation within the defined autoregulatory range (55–95 mmHg). For sample size comparing GPR39 KO vs WT, power was calculated at n = 11 to be 80% with type I error 5% using a Wilcoxon Test assuming difference in means of 0.008 for standard deviation 0.007 in one group and 0.005 in another.

For each individual animal, univariable linear regression was performed on the data points collected across the predefined autoregulatory range. The resulting slope coefficient (β_1) from this regression ($CBF = \beta_0 + \beta_1 CDP$) was used as a single data point representing that animal's degree of autoregulation. A slope near zero indicates successful autoregulation, while a positive slope indicates a pressure-dependent relationship.

Non-parametric statistical tests were used for all group comparisons to avoid assumptions of normal data distribution. For comparisons between two independent groups the Mann-Whitney U Test was used.

For comparisons between three or more independent groups the Kruskal-Wallis Test was used and Dunn's test for multiple comparisons was performed as a post-hoc analysis to determine which specific group pairings were significantly different and is denoted with p_D in the results. Mean $\pm$ 1SEM slope in the autoregulatory range of CDP is calculated within each group of mice. A p-value of less than 0.05 was considered statistically significant for all tests. All statistical analyses were conducted using R v4.5.1. Figures were produced with GraphPad Prism v10.0

Results

It was previously shown that isolated rodent hearts perfused with mKHB or crystalloid cardioplegia solutions do not exhibit autoregulation [5–9]. Accordingly, we perfused 9 WT mouse hearts with mKHB over a wide range of CDPs and found no autoregulation (Fig. 2A, mean slope 0.0186 ± 0.0022). Further, it was suggested that adding plasma or blood to the Krebs's solution resulted in autoregulation [5–9]. We, therefore, mixed plasma (10% v/v bovine plasma with heparin HemoStat laboratories, Dixon, CA, USA) with mKHB, allowed adequate oxygenation, and then perfused the heart in 7 animals for 5 min at 80 mmHg before altering CDP. We noted classic autoregulation with flattening of CBF between 55 and 95 mmHg (Fig. 2B; mean slope 0.0066 ± 0.0016 ; $p_D=0.0012$ compared to mKHB).

Since albumin in plasma binds to many moieties, including fatty acids that have been demonstrated to be vasoactive [17–21] we first tested whether adding 3% fatty acid-free albumin (FAFA, Millipore Sigma, St. Louis, MO, USA) to mKHB would result in autoregulation. Eight animals were perfused with mKHB with 3% FAFA administered via the sideline at 80 $\mu\text{L}/\text{min}$ for 5 min at each CDP. Again, no autoregulation was noted over a wide range of CDPs (Fig. 2C; mean slope 0.0160 ± 0.0008 , $p_D=0.45$ compared to mKHB and $p_D=0.0011$ compared to mKHB + 10% plasma).

AA is the major FA in plasma and has been shown to be vasoactive [19–21]. Hence, we infused various doses of AA (Nu-Chek Prep, Inc. Elysian, MN, USA) to determine the concentration required to produce coronary autoregulation. Mice were randomized to receive incremental doses of AA (1, 4, 8, or 16 nmol) dissolved in 3% FAFA, administered via the side port over 5 min at a rate of 80 $\mu\text{L}/\text{min}$, while the heart was perfused with mKHB. Figure 3 illustrates the results. Lower doses of AA did not produce autoregulation (panels A to C, mean slopes 0.0151 ± 0.0017 , 0.0136 ± 0.0016 , 0.0126 ± 0.0018 , respectively). Autoregulation was seen after 5 min of treatment with 16 nmol AA (panel D; mean slope 0.0086 ± 0.0011 ; $p_D=0.021$ compared to 1 nmol AA; Kruskal-Wallis global p-value across doses = 0.036. This converts to 3.2 nmol/min (15 nmol/5 min), which was the continuous infusion rate used for subsequent experiments.

We then used the combination of mKHB, 3% FAFA and 3.2 nmol/min of AA (the last 2 infused via the side needle at 80 $\mu\text{L}/\text{min}$) over a wide range of CDP in 10 WT and 8 GPR39 KO mice. The WT mice showed classic autoregulation (Fig. 4A; mean slope 0.0088 ± 0.0015) but the GPR39 mice did not (Fig. 4B; mean slope 0.0184 ± 0.0022 ; $p_D=0.0044$ comparing WT with GPR39). GPR39 is the target of the AA derivative, 15-HETE, implying that it participates in coronary autoregulation.

AA is released from plasma membrane in the heart by cytosolic PLA₂ [22,23]. We, therefore, blocked its action by adding the specific inhibitor, palmitoyl trifluoromethyl ketone (PACOCF₃, Abcam, Cambridge, UK) at 2 concentrations (10 μM and 50 μM) to the mKHB solution.²⁴ PACOCF₃ was dissolved in dimethyl sulfoxide (DMSO) before mixing with mKHB (final concentration of DMSO was 0.1%). Via the side-line we infused 3% FAFA combined with 3.2 nmol AA/min at 80 μL/min. Six animals each were used for the 2 PACOCF₃ doses while 7 animals were used for just 0.1% DMSO in mKHB (vehicle). Animals were randomized to vehicle versus drug (PACOCF₃). The results are shown in Fig. 5. Autoregulation is abolished at both doses of PACOCF₃ (panels A and B; mean slope 0.0228 ± 0.0026 in 10 μM, 0.0243 ± 0.0032 in 50 μM), while it is retained when only DMSO is infused (panel C; mean slope 0.0120 ± 0.0034 ; $p_D=0.033$ compared to 10 μM; $p_D=0.051$ compared to 50 μM).

Ambient microvascular arterial pressure sensing is an integral part of autoregulation. Accordingly we used 2 concentrations (5 nM and 10 nM) of 2-Aminoethoxydiphenyl borate (2-APB, Millipore Sigma, St. Louis, MO, USA) a non-specific blocker of the transient receptor potential (TRP) channels, store operated Ca⁺⁺ channels (SOCs), and inositol 1,4,5-trisphosphate (IP3) receptor to inhibit potential sources of intraluminal pressure sensing in the coronary system [25–28]. Via the sideline we infused a combination of 3% FAFA and 3.2 nmol AA at a rate of 80 μL/min. 2-APB was dissolved in DMSO before mixing with mKHB. Animals were randomized to the two doses and DMSO. Figure 6 shows that 2-APB almost abolished autoregulation (Panels A and B; mean slope 0.0211 ± 0.0022 in 5 μM, 0.0206 ± 0.0021 in 10 μM, $p = 0.0989$), while vehicle (DMSO) did not (panel C; mean slope 0.0120 ± 0.0034 ; global Kruskal-Wallis $p = 0.0989$, $p_D=0.0806$ compared to 5 μM; $p_D=0.0525$ compared to 10 μM). Higher doses were not used because they cause atrial fibrillation.²⁸ Since the vehicle was the same for both PACOCF₃ and 2-APB, panel C in the same in Figs. 5 and 6.

Discussion

The new information from this study is that AA and GPR39 participate in the in vivo myogenic response that forms the molecular basis of coronary autoregulation. When pressure is registered in the coronary arterial system through mechanotransduction, it activates cytosolic PLA₂, releasing AA from the plasma membrane that is then converted into its metabolites, including 15-HETE. 15-HETE activates GPR39 in VSMCs, increasing cytosolic Ca⁺⁺ and causing VSMC contraction, the degree of which is commensurate with the pressure sensed within the system. Thus, when pressure is high more 15-HETE is released and more vasoconstriction occurs. When pressure is low, less 15-HETE is released, and less vasoconstriction occurs (vasodilation in relative terms). We have previously shown that within the autoregulatory range, 15-HETE levels decline with declining CDP [12]. We have also shown that pharmacological inhibition of GPR39 abolishes autoregulation and CBF because CDP dependent [12]. Here we show that genetic deletion of GPR39 does the same. The graphical abstract illustrates the proposed molecular mechanism of coronary autoregulation based on our results.

In this study we show that specific inhibition of PLA₂ that ostensibly precludes release of AA from cell membrane also abolishes autoregulation. Further, nonspecific inhibition of TRP and SOC channels as well as IP3 also reduces autoregulation presumably by preventing mechanotransduction of intraluminal vascular pressure. The aim of this study was not to identify the specific mechanosensing ion channels or G-protein coupled receptors but to demonstrate that interrupting mechanotransduction abolishes autoregulation. Identifying the specific channel(s) or G-protein coupled receptors participating as mechanosensors in coronary autoregulation is beyond the scope of the present study.

Our study clearly shows that use of mKHB is not conducive to coronary physiology in the mouse. Others have previously reported similar results in mice and other rodents [5–9]. Thus, unless plasma, blood, or AA is present in the perfusate autoregulation does not occur in these animals. These findings question the in vivo relevance of the in vitro observed myogenic response as an inherent VSMC property. It rather seems that although VSMC contractile machinery and GPR39 participate in the myogenic response and autoregulation, autoregulation in the mouse does not occur in the absence of AA, which presumably precludes bioavailability of 15-HETE and the associated vasomotor response.

Critique of the study

There are other fatty acids in plasma in addition to AA. We selected AA because of its abundance and known vasoactive properties [19–21]. Other, less abundant fatty acids could possibly participate in autoregulation through other metabolites and receptors [17, 18]. There is scant literature on the fate of AA in VSMCs during mKHB infusion. Our results, and that of others [5, 6], would indicate that AA release from plasma membrane is inhibited during mKHB delivery necessitating supplemental AA to elicit autoregulation.

We did not address one step in 15-HETE production from AA – 15-LO. It is possible that 15-LO may respond to mechanotransduction signals but we could not find anything in the literature supporting this. Further, we could not find any reference to 15-LO during cardioplegia. We were also unable to reliably measure 15-HETE or AA in the heart effluent because of undetectable or very low concentrations.

Zn⁺⁺ is an allosteric modulator of GPR39 [15]. mKHB does not contain Zn⁺⁺ while plasma does, which might partially explain the autoregulation seen with plasma in our as well as previous studies [5–9]. We reported activation of GPR39 by 15-HETE at 100 nM concentration in the absence of Zn⁺⁺, while adding 4 μM Zn⁺⁺ allowed GPR39 activation at an order of magnitude lower concentration [13]. The direct effects of Zn⁺⁺ itself on GPR39 were reported at supraphysiological doses [14].

Selecting the dose for specific enzyme blockers can be difficult. For instance, PACOCF₃ inhibits both Ca⁺⁺ dependent and independent PLA₂ with reported IC₅₀ values of 45 μM and 3.8 μM, respectively. This is the reason we used 2 concentrations of the drug and found both to be effective in abolishing autoregulation. Similarly, 2-APB not only inhibits TRP channels, but also inhibits the IP3 receptor

(intracellular Ca^{++} releasing channel) and SOCs, thereby interfering with Ca^{++} signaling. Ca^{++} signaling via these channels could play a role in mechanotransduction [29–31]. 2-APB almost abolished autoregulation at the doses used. We did not use higher doses because they are reported to cause atrial fibrillation. Since 2-APB does not block arachidonate-regulated Ca^{++} (ARC) channels [32], these channels likely do not participate in autoregulation.

Early studies using isolated heart preparations showed that crystalloid solutions abolished autoregulation unless pyruvate (2 mM) was added to the solution [33, 34]. Pyruvate activates PLA_2 , releasing AA [35, 36]. While mKHB used in our study has the required concentration of pyruvate, we did not observe autoregulation when mKHB was used alone. In previous studies, where it has been successfully used to study coronary flow-function relations [32, 37, 38], the animal species ranged from rat to cat. It is quite possible that in mouse even adding 2mM pyruvate to a crystalloid solution is not enough to stimulate PLA_2 to release of sufficient amounts of AA to elicit autoregulation, requiring supplemental AA.

Although our study was not designed to determine the specific ion channel or G-protein coupled receptor [39–41] for mechanotransduction of arteriolar pressure for autoregulation, it is interesting to note that AA and other lipid molecules such as phosphatidylinositol biphosphate (PIP2) sensitize certain TRP channels to mechanotransduction [30, 31]. The coupling of AA, Ca^{++} signaling, and mechanotransduction is very ancient and conserved in various species [42]. Consequently, it should not be surprising that this triad forms the basis of a teleologically old phenomenon such as coronary autoregulation.

Conclusions

Arachidonic acid and GPR39 form the molecular basis of vivo myogenic response and coronary autoregulation. Advent of pharmacological agents based on this understanding of coronary autoregulation could improve the management of patients with coronary artery disease.

Abbreviations

AA arachidonic acid

2-APB 2-aminoethoxydiphenyl borate

ARC arachidonate-regulated Ca^{++} channels

15-HETE 15-hydroxyeicosatetraenoic acid

15-LO 15-lipoxygenase

AA arachidonic acid

CBF coronary blood flow

CDP coronary driving pressure

DMSO dimethyl sulfoxide

FAFA fatty acid free albumin

Gaq G-protein α_q

GPR39 G-protein coupled receptor

IP3 inositol 1,4,5-trisphosphate

mKHB modified Krebs-Henseleit buffer

PACOCF₃ palmitoyl trifluoromethyl ketone

PIP phosphatidylinositol biphosphate

PLA₂ phospholipase A₂

TRP transient receptor potential

SOC store-operated calcium channel

VSMC vascular smooth muscle cell

Declarations

Conflict of Interest

There is no conflict of interest for any of the authors.

Author Contribution

Drs. Kajimoto, Methner and Kaul developed the concept and design for the studies. Dr. Kajimoto performed all the experiments and curated the data. Drs Kajimoto, Methner and Kaul reviewed weekly progress. Dr. Kajimoto and Methner made the Figures. Dr. Minnier performed the statistical analysis. Drs Kajimoto and Kaul wrote the paper. All authors have reviewed the final version.

Acknowledgement

Data Availability

The data that support the findings of this study are available from the corresponding author, Sanjiv Kaul, MD, upon reasonable request.

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Figures

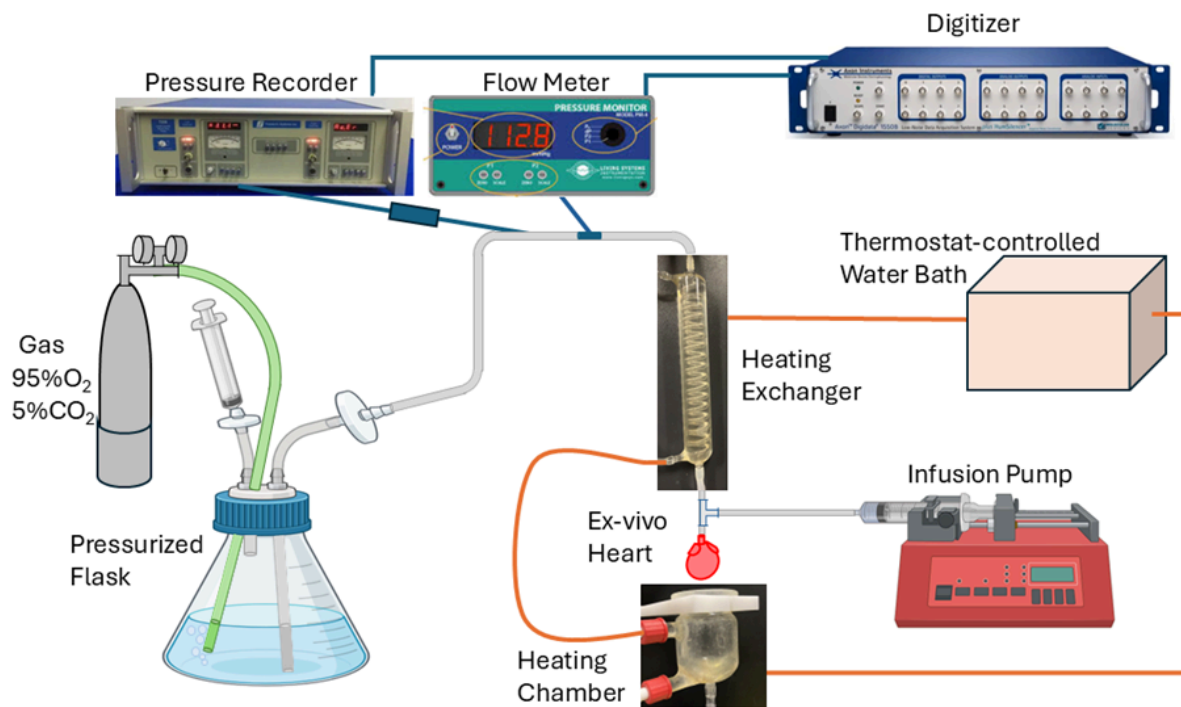


Figure 1

Experimental Setup. After mouse aorta was cannulated, it was perfused with modified Krebs-Henseleit buffer (mKHB) using a closed circulatory system where mean coronary pressure could be adjusted from 30 to 140 mmHg by pressurizing the flask containing the perfusate. System pressure and flow were measured by placement of an in-line flow probe and a pressure transducer that were connected to a flow meter and a pressure recorder. These were interfaced to a digitizer for data recording and analysis. A needle was connected to the cannula feeding the aorta through which chemicals and drugs could be administered using an infusion pump. The temperature of perfusate was controlled using a heating bath.

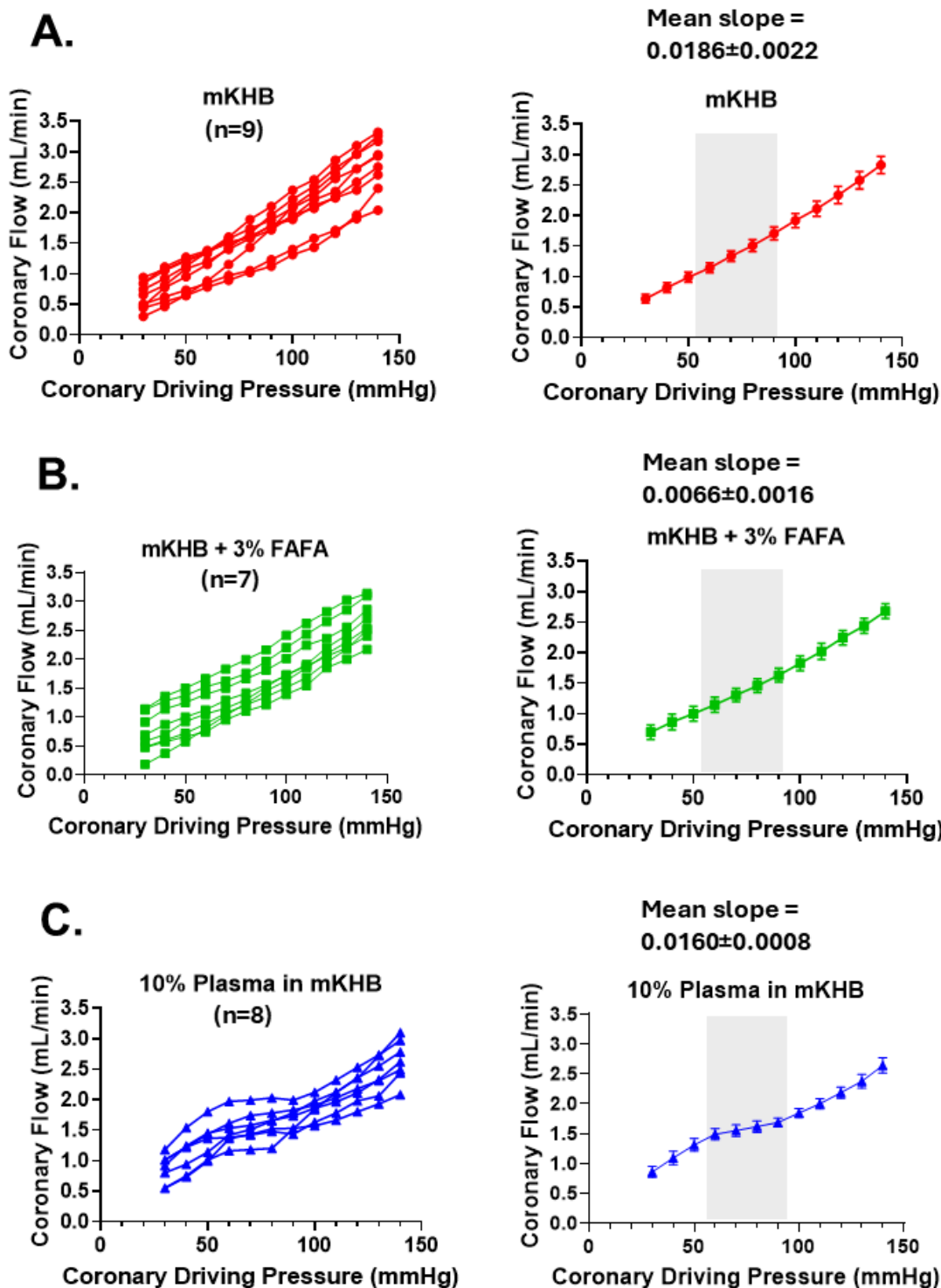


Figure 2

Effects on coronary flow-pressure relations of A) mKHB, B) nKHB + FAFA, C) mKHB + Plasma

Effect of perfusing different solutions on coronary pressure-flow relations. Panels to the left show data derived from individual animals and panels to the right depict aggregate data presented as mean ± 1 SEM. A): When heart was perfused with modified Krebs-Henseleit buffer (mKHB) a linear relation

between pressure and flow was noted ($n=9$, mean slope 0.0186 ± 0.0022). B) When bovine plasma was added to mKHB making it into a 10% plasma solution ($n=7$) there was flattening of the pressure-flow relation between approximately 55 and 95 mmHg, exemplifying autoregulation (mean slope 0.0066 ± 0.0016 ; $p_D=0.0012$ compared to mKHB). C) When, in the presence of mKHB, 3% fatty acid free albumin (FAFA) was infused into the heart through the side-line ($n=8$) at a rate of $80 \mu\text{L}/\text{min}$, no autoregulation was noted (mean slope 0.0160 ± 0.0008 , $p_D=0.0011$ compared to mKHB+plasma, $p_D=0.45$ compared using Kruskal-Wallis test followed by Dunn's test for multiple comparisons). These results imply that non albumin component of plasma is required for coronary autoregulation.

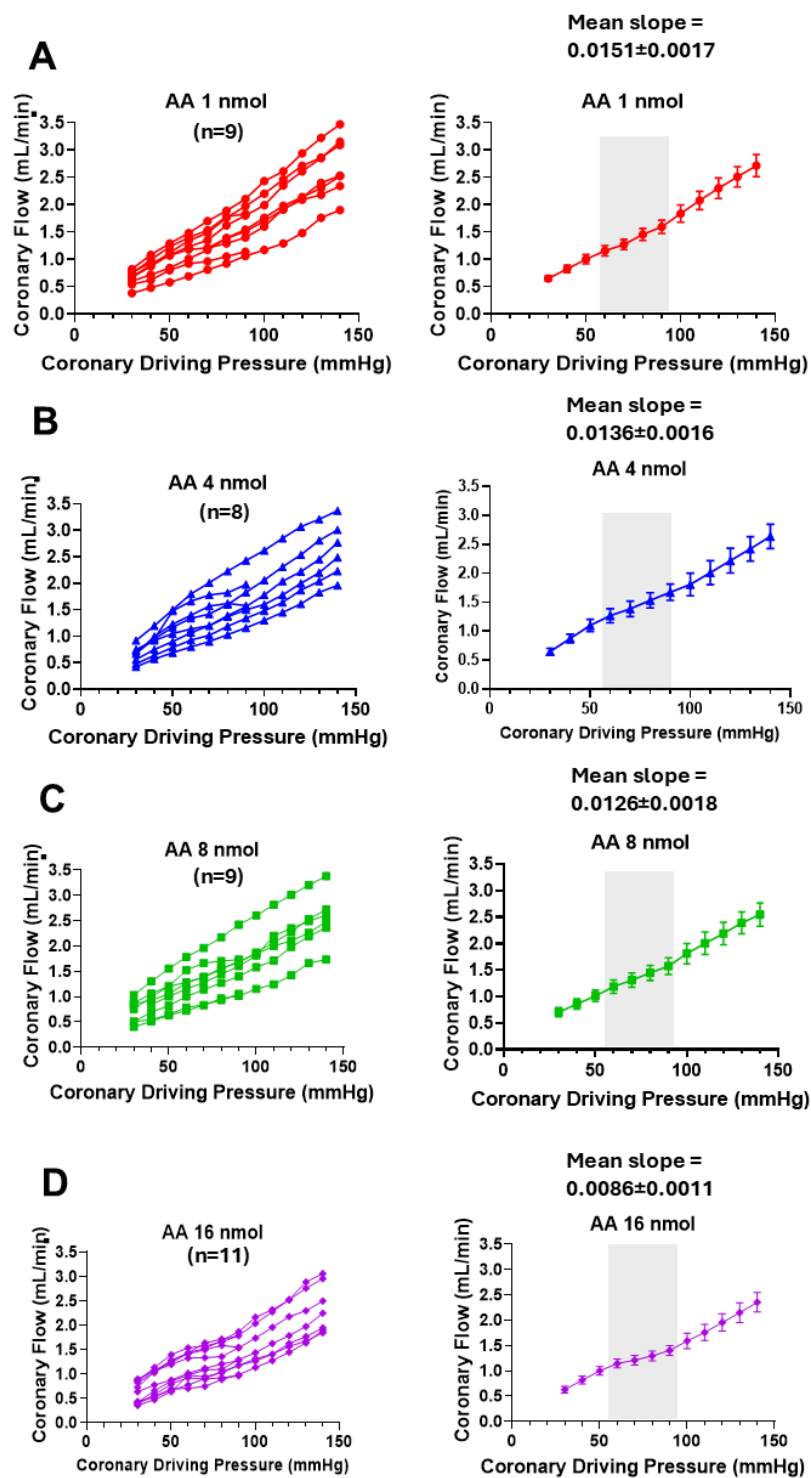


Figure 3

Effects on coronary flow-pressure relations of A) mKHB, B) nKHB + FAFA, C) mKHB + Plasma

Effect of altering coronary driving pressure on coronary blood flow in the presence of different concentrations of arachidonic acid (AA). Heart is perfused with modified Krebs-Henseleit buffer (mKHB) containing different concentrations of AA with 3% FAFA administered via the side-line over 5 min at

which time data were acquired. Panels on the left depict data derived from individual animals and panels on the right show aggregate data mean±1SEM results. The relation between coronary pressure and coronary blood flow is linear at doses of 1 nmol (A, n=9, mean slope 0.0151±0.0017), 4 nmol (B, n=8, mean slope 0.0136±0.0016), and 8 nmol (C, n=9, mean slope 0.0126±0.0018). However, the relation flattens between 55 to 95 mmHg, when 16 mol of AA (n=11) is used (D) exemplifying coronary autoregulation (mean slope 0.0086±0.0011; $p_D=0.021$ compared to 1 nmol by Kruskal-Wallis test (global p value of 0.036 across all doses) followed by Dunn's test for multiple comparisons). These results indicate that AA participates in coronary autoregulation.

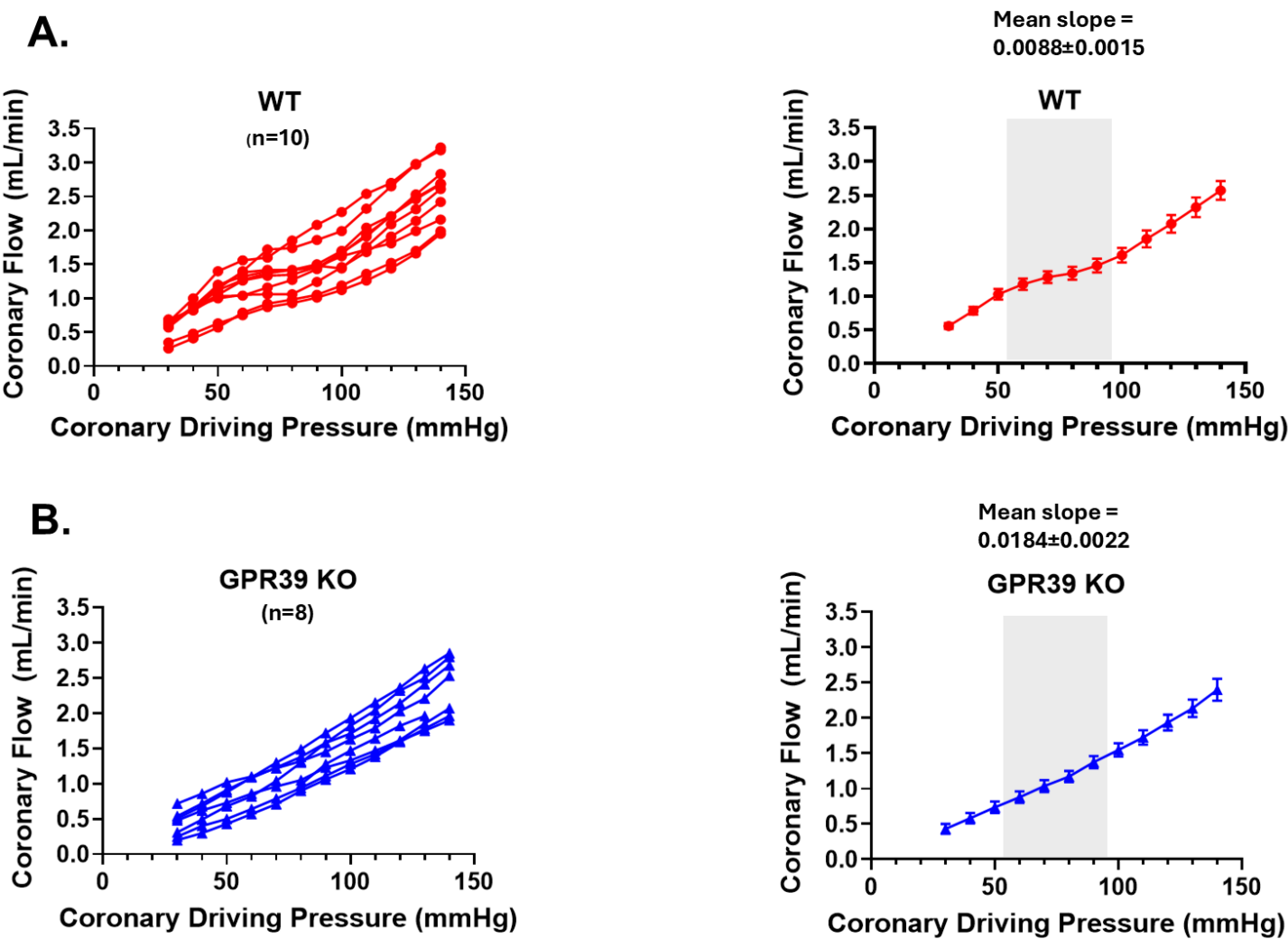


Figure 4

Effects on coronary flow-pressure relations of mKHB +AA in A) wild-type and B) GPR knockout mice

Effect of altering coronary driving pressure on coronary blood flow in wild type (WT) and GPR39 KO mice. Heart is perfused with modified Krebs-Henseleit buffer (mKHB) at different pressures and 3% FAFA combined with 3.2 nmol of AA are infused via the side-line at 80 μ L/min. Panels on the left show data derived from individual animals and panels on the right depict aggregate data presented as mean $\pm$ 1SEM. When wild-type animals (n=10) receive this mixture, they exhibit autoregulation (A, mean slope

0.0088±0.0015). In contrast when this mixture is given to GPR39 knockout mice (n=8) no autoregulation is noted (mean slope 0.0184±0.0022; $p_D=0.0044$ by Mann-Whitney U Test comparing WT with GPR39). These results imply that GPR39, a target for 15-HETE, which is a metabolite of AA, participates in coronary autoregulation.

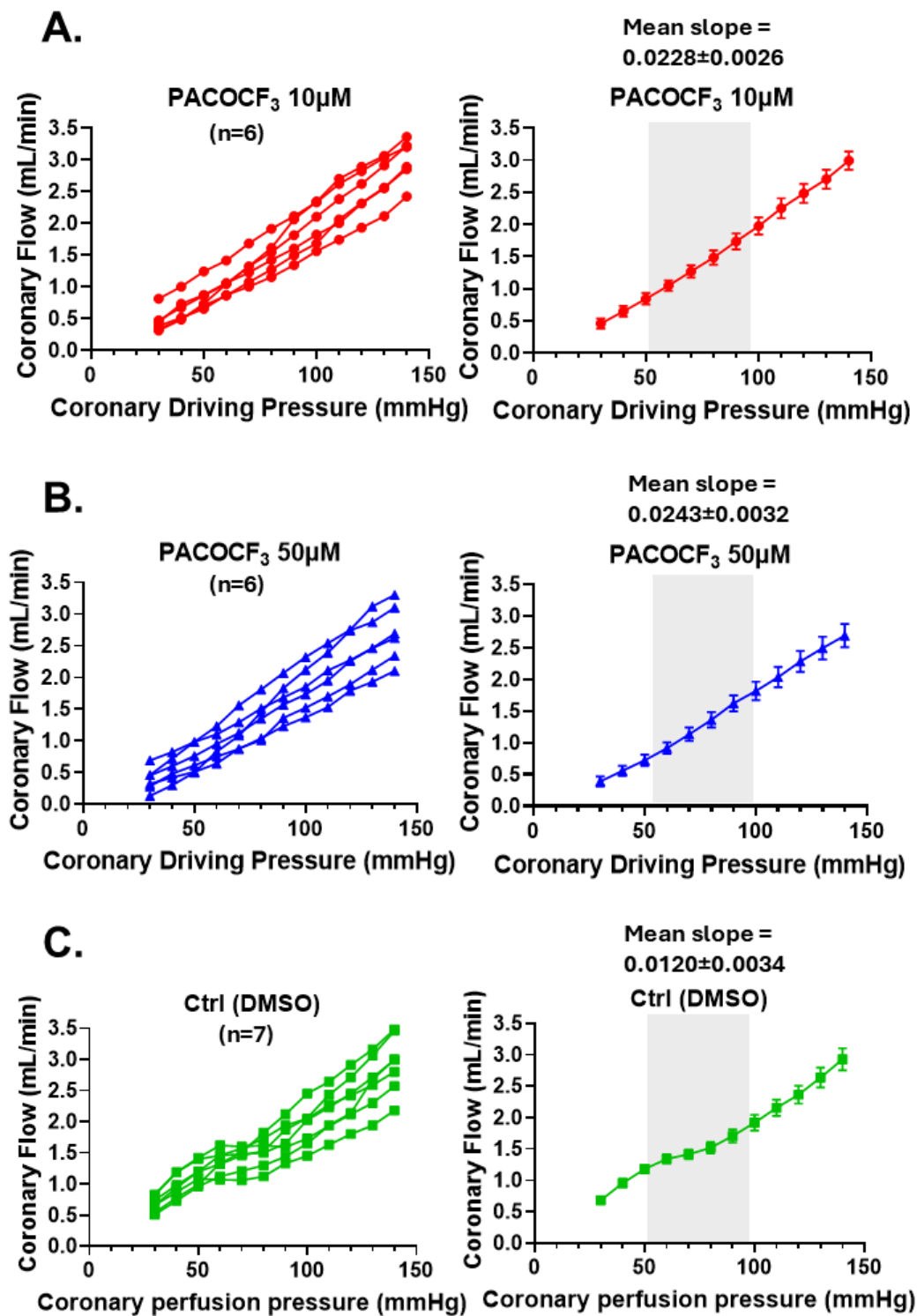


Figure 5

Effects on coronary flow-pressure relations of mKHB +AA in with different doses of PACOCF₃ and vehicle

Effect of altering coronary driving pressure on coronary blood flow in the presence of cytosolic phospholipase A₂ (PLA₂) inhibitor palmityl trifluoromethyl ketone (PACOCF₃). PACOCF₃ (dissolved in DMSO) was mixed with modified Krebs-Henseleit buffer (mKHB) at 2 concentrations. Vehicle comprised 0.1% dimethyl sulfoxide (DMSO). Panels on the left show data derived from individual animals and panels on the right depict aggregate data presented as mean $\pm$ 1SEM. When 10 μ M of PACOCF₃ is given to wild-type mice (n=6), no autoregulation is noted (A). Similar results are obtained when 50 μ M of PACOCF₃ (n=6) is given (B) (mean slope 0.0228 ± 0.0026 in 10 μ M, 0.0243 ± 0.0032 in 50 μ M). In comparison, when vehicle (0.1% DMSO) is given (n=7), autoregulation is noted (C) (mean slope 0.0120 ± 0.0034 ; $p_D=0.033$ compared to 10 μ M; $p_D=0.051$ compared to 50 μ M by Kruskal-Wallis test followed by Dunn's test for multiple comparisons). PLA₂ releases AA from cell membranes before it can be converted to 15-HETE. These results indicate that inhibiting PLA₂ prevents bioavailability of AA that participates in coronary autoregulation.

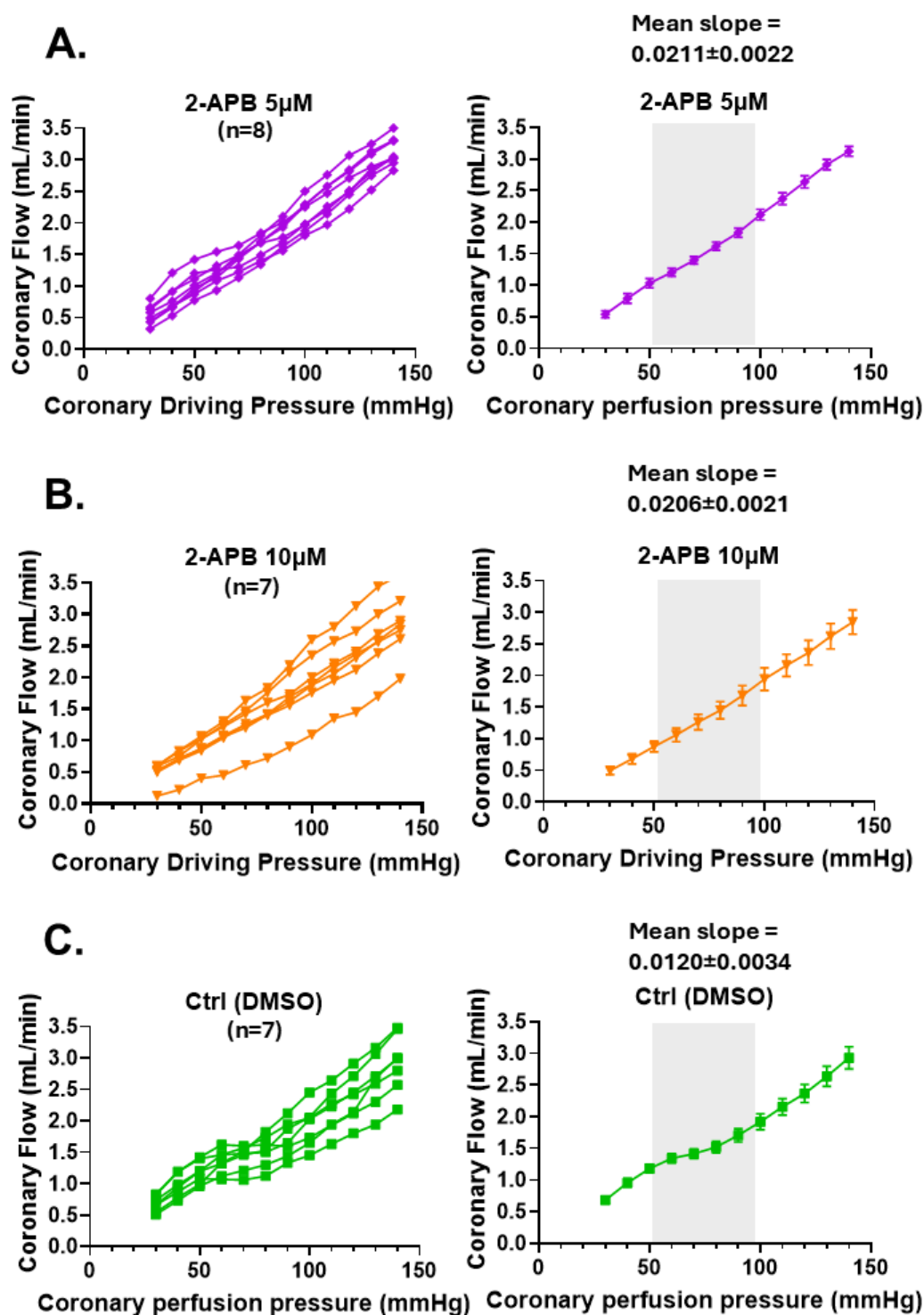


Figure 6

Effects on coronary flow-pressure relations of mKHB +AA in with different doses of 2-APB and vehicle

Effect of altering coronary driving pressure on coronary blood flow in the presence of a non-specific transient receptor potential (TRP) channel inhibitor, 2-aminoethoxydiphenyl borate (2-APB). 2APB, dissolved in dimethyl sulfoxide (DMSO), was mixed with modified Krebs-Henseleit buffer (mKHB) at 2

concentrations. Vehicle comprised 0.1% DMSO. Panels to the left show data derived from individual animals and panels to the right illustrate aggregate data presented as mean $\pm$ 1SEM. When 5 μ M of 2-APB is given to wild-type mice (n= 8), less autoregulation is noted (A). Similar results are obtained when 10 μ M (n=7) is given (B) (mean slope 0.0211 ± 0.0022 in 5 μ M, 0.0206 ± 0.0021 in 10 μ M). Infusion of vehicle (DMSO, n=7, data duplicated from Figure 5) shows autoregulation not (panel C; mean slope 0.0120 ± 0.0034 ; global Kruskal-Wallis $p=0.0989$, $p_D=0.0806$ compared to 5 μ M; $p_D=0.0525$ compared to 10 μ M).

Supplementary Files

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