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Inhibition of soluble epoxide hydrolase attenuates endothelial dysfunction in animal models of diabetes, obesity and hypertension

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ABSTRACT

Endothelial dysfunction is a hallmark of, and plays a pivotal role in the pathogenesis of cardiometabolic diseases, including type II diabetes, obesity, and hypertension. It has been well established that epoxyeicosatrienoic acids (EETs) act as an endothelial derived hyperpolarization factor (EDHF). Soluble epoxide hydrolase (s-EH) rapidly hydrolyses certain epoxylipids (e.g. EETs) to less bioactive diols (DHETs), thereby attenuating the evoked vasodilator effects. The aim of the present study was to examine if inhibition of s-EH can restore impaired endothelial function in three animal models of cardiometabolic diseases. Isolated vessel rings of the aorta and/or mesenteric artery from mice or rats were pre-contracted using phenylephrine or U46619. Endothelium-dependent and independent vasorelaxation to acetylcholine and sodium nitroprusside (SNP) were measured using wire myography in vessels isolated from db/db or diet-induced obesity (DIO) mice, and angiotensin II-induced hypertensive rats treated chronically with s-EH inhibitors AR9281 or AR9276 or with vehicle. Vasorelaxation to acetylcholine, but not to SNP was severely impaired in all three animal models. Oral administration of AR9281 or AR9276 abolished whole blood s-EH activity, elevated epoxy/diol lipid ratio, and abrogated endothelial dysfunction in all three models. Incubating the mesenteric artery of db/db mice with L-NAME and indomethacin to block nitric oxide (NO) and prostacyclin formation did not affect AR9821-induced improvement of endothelial function. These data indicate that inhibition of s-EH ameliorates endothelial dysfunction and that effects in the db/db model are independent of the presence of NO and cyclooxygenase derived prostanoids. Thus, preserving vasodilator EETs by inhibition of s-EH may be of therapeutic benefit by improving endothelial function in cardiometabolic diseases.

1. Introduction

Endothelial dysfunction, manifest as impaired endothelium-dependent vasorelaxation to stimuli such as acetylcholine or shear stress, is a hallmark of, and plays a pivotal role in pathogenesis of cardiometabolic diseases, including type II diabetes, obesity, and hypertension. Endothelium-dependent vasorelaxation is mediated by endothelium-derived relaxing factors (EDRF), comprising a diverse group of autacoids including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) (Esper et al., 2006). EDHF is an operational term describing a range of endothelium-derived modalities including epoxyeicosatrienoic acids (EETs), hydrogen peroxide (H_2O_2), potassium ions (K^+), C-type natriuretic peptide (CNP) which share a common property of hyperpolarizing vascular smooth muscle. The relative contribution of each of these mediators to endothelium-dependent vasorelaxation is inversely related to vessel caliber, such that NO- and prostacyclin-mediated responses predominate in large conduit vessels, whereas EDHF responses are more prominent in small resistance arteries (Xu et al., 2007). Because the resistance vessels are the major contributors to peripheral resistance and tissue perfusion, EDHF is likely to play a pivotal role in vascular homeostasis and its functional impairment represent an important component of mechanisms for cardiometabolic disease (Feletou and Vanhoutte, 2006).

EETs, metabolites of arachidonic acid generated in vascular endothelial cells by various cytochrome P450 (CYP) epoxygenase enzymes, are believed to function as an EDHF (Campbell and Falck, 2007; Inceoglu et al., 2007). EETs are degraded into corresponding less biologically active diols, dihydroeicosatrienoic acids (DHETs), via the activity of a ubiquitous enzyme, soluble epoxide hydrolase (s-EH) (Campbell and Falck, 2007; Inceoglu et al., 2007). Inhibition of s-EH prevents EET degradation, and enhances their biological activities. Data from animal studies have shown that s-EH inhibitors possess anti-hypertensive and glucose lowering benefits (Imig, 2006; Wong et al., 2009). There is, however, a paucity of data exploring the effects

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of s-EH inhibitors on endothelial dysfunction in animal models of cardiometabolic diseases. Therefore, the aim of the present study is to test the hypothesis that inhibition of s-EH can improve endothelial function in three animal models of cardiometabolic diseases known to manifest endothelial dysfunction, including diabetic db/db mice, diet-induced obese (DIO) mice and angiotensin II-induced hypertensive rats.

2. Materials and methods

2.1. Animals and experimental design

Animals were given free access to food and water, and housed in a single case in a room with a constant temperature of 22 °C on a 12:12 light–dark cycle (0700–1900 h). All protocols were approved by the Institutional Animal Care and Use at Murigenics, Inc.

2.2. Diabetic db/db mice

Seven-week old male diabetic mice (*Mus musculus*, genotype db/db) were purchased from The Jackson Laboratory (Bar Harbor, ME.). After one week acclimatization, mice were fasted for ~6 h and the fasting blood glucose levels were measured from tail cuts using a glucometer. Based on the pre-dose fasting blood glucose levels, mice were randomized for assignment into two groups of ten mice each. The drinking water was then replaced with either vehicle (5% hydroxypropyl cyclodextrin, HPCD) or AR9281 at 1.5 g/l for 8 weeks. The calculated daily dose based on the daily liquid consumption was 383 mg/kg. After 8-weeks of treatment, the animals' fasting glucose levels were measured again, and then the animals were sacrificed. Blood samples were collected via cardiac puncture and the levels of the arachidonic acid metabolites, EETs and DHETs, and the linoleic acid metabolites, epoxyoctadecanoic acid (EpOME) and dihydroxyoctadecanoic acid (DiHOME), were measured for evaluation of s-EH activity as described in the section below. Animals were euthanized and the descending thoracic aorta and a second branch of mesenteric artery were dissected and used for wire myography.

2.3. High fat and fructose diet-induced obese (DIO) mice

Eight-week old male C57 BL/6 mice, purchased from Charles River Laboratories (Hollister, CA), were fed a regular rodent chow (Purina Rodent Diet 20 5053) or a high-fat and fructose (HFF) diet consisting of the 45% fat (Diet no. D12451, Research Diets, New Brunswick, NJ) and degassed 7UP as drinking water (Cadbury Schweppes, Plano, TX). After ten weeks, 10 chow-fed mice and 10 DIO mice were dosed with vehicle (1% carboxymethyl cellulose, CMC + 0.1% tween 80 in water), and another 10 DIO mice with AR9281 at 100 mg/kg, all via oral gavage twice daily. After four-weeks of treatment, animals were sacrificed 6–10 h following the last dose. Blood samples were collected via cardiac puncture for measurement of glucose level and s-EH activity. Animals were euthanized and the descending thoracic aorta and a second branch of the mesenteric artery were dissected and used for wire myography.

2.4. Angiotensin II-induced hypertensive rats

Male Sprague–Dawley (SD) rats, 8–10 weeks old, purchased from Charles River Laboratories (Hollister, CA), were surgically implanted subcutaneously with Angiotensin II-containing Alzet® pumps, along with hemodynamic telemetry probes (Data Sciences International, St. Paul, MN). The pump provided a continuous subcutaneous infusion of AngII at 100 ng/min. After 8–10 days of angiotensin II infusion, blood pressure and heart rate were measured by telemetry, and 18 rats with hypertension were divided into three groups. From this point, rats were provided ad libitum with tap water with or without 0.3 g/l AR9281 or 0.1 g/l AR9276 for 14 days. Based on daily water consumption, the calculated daily doses of AR9281 and AR9276 are ~30 and ~10 mg/kg, respectively. The animals were then euthanized and the descending thoracic aortas were dissected for use in myography.

2.5. The assays for blood sEH activity and plasma concentrations of EpOMEs and DiHOMEs

The assay for s-EH activity in whole blood was modified from work by Seidegard et al. (1984). In the assay, the exogenous substrate, 14, 15-EET, was added to the blood samples as the probe substrate; formation of the hydrolysis product, 14, 15-DHET was presumed to be mediated primarily by s-EH. To determine background activity (i.e., in the absence of s-EH), the known s-EH inhibitor 12-(3-adamantyl-ureido)-dodecanoic acid was added to the identical control reactions that were run in parallel.

Plasma samples were extracted for measurement of EpOMEs and DiHOMEs using ethyl acetate via liquid–liquid extraction as described previously (Newman et al., 2002). The organic layer was transferred to a 96-well collection plate, dried down using inert gas, then reconstituted with twice of the original plasma volume of methanol: water (50:50). 10 μl samples were injected onto a Gemini C18 column (2.0 × 20 mm, 3 μm) in a gradient run with a mobile phase consisting of water and methanol/acetonitrile containing 0.2% acetic acid. Each EpOME/DiHOME pair was quantified in a single run by negative mode electrospray ionization with tandem quadrupole mass spectroscopy. Calibration standards were made freshly by diluting stock solutions of the respective species in methanol:water.

2.6. Myography

Following euthanasia, a thoracolaparotomy was performed. The mesenteric arterial branches and descending thoracic aorta were excised and placed in Petri dishes containing room temperature physiological salt solution (PSS) with the following composition (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 11.1 glucose. The vessels were carefully cleaned of loose connective tissue under a dissecting microscope, and 3 mm aortic segments and 2 mm second-order mesenteric arterial branches were cut and mounted in the myograph. The myograph chambers (Multi Myograph System-610M; DMT-USA, Inc., Sarasota, FL) were filled with PBS held at 37 °C and gassed with 95% O_2 and 5% CO_2 at pH 7.4. Mechanical activity was recorded isometrically by a force transducer coupled to a Powerlab data acquisition system (AD-Instruments, Colorado Springs, CO). The tissues were incubated at 37 °C, gassed with 95% O_2 and 5% CO_2, and suspended isometrically under resting tensions of 1 g for aorta and 0.1–0.2 g for mesenteric artery branches. All tissues were initially equilibrated for 1.5 h. During the incubation period, the loading tensions were adjusted periodically and maintained throughout the equilibration period. The incubation media were routinely changed every 15 min as a precaution against interfering metabolites (Altura and Altura, 1970). The concentration–relaxation response curves to acetylcholine (from 1 nM to 10 μM) and sodium nitroprusside (SNP, from 1 nM to 10 μM) were performed in rings pre-contracted by phenylephrine (0.1–1 μM) for the aorta or U46619 (0.1–1 μM) for the mesenteric arterial branches, which evoke a sub-maximal constriction, ~12–14 mN/mm in rat aorta and 3 mN/mm in mice mesenteric arterial branches. The acetylcholine concentration–relaxation response curves were additionally performed in rings pre-exposed to NG-nitro-L-arginine methyl ester (L-NAME, 100 μM), an inhibitor of nitric oxide formation in combination with indomethacin (10 μM), an inhibitor of cyclooxygenase. For relaxation response curves, forces were normalized as percent of pre-contraction values. Maximal response (E_{max}) and sensitivity (pD_2: − log molar concentration of the drug required to produce 50% of the maximum response) were

determined by computer-assisted interactive nonlinear regression analysis (GraphPad Prism, San Diego, Calif., USA).

2.7. Compounds and chemicals

AR9281 (1-(1-acetyl-piperidine-4-yl)-3-adamantan-1-yl-urea) and AR9276 (1-(1-nicotinoylpiperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)urea) are potent, selective and orally available s-EH inhibitors, discovered by Arete Therapeutics, Inc. The detailed physiochemical and pharmacological properties of the compounds are published separately (Wong et al., 2009; Zhang et al., 2009). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) except for U46619 (Cayman, Ann Arbor, MI). Stock solutions of compounds or chemicals were prepared in distilled water except for indomethacin (dissolved in distilled water containing 4% $NaHCO_3$ and sonicated before use). Preliminary experiments established that none of the vehicles exerted any significant vascular effects.

2.8. Statistics

All results are presented as the mean and standard error (S.E.M.) for the number of animals (n) indicated. Multiple comparisons of mean values were performed by analysis of variance (ANOVA) followed by a subsequent Student–Newman–Keuls test for repeated measures. Differences were considered to be statistically significant when the P value was <0.05.

3. Results

3.1. AR9281 enhanced endothelium-dependent vasorelaxation in the mesenteric arteries but not aorta of diabetic db/db mice

AR9281 administered in drinking water (383 mg/kg daily dose) completely abolished whole blood s-EH activity measured by the conversion rate of exogenously added 14, 15 EET to 14, 15 DHET (Table 1). Consequently, the plasma EpOME/DiHOME ratios were significantly elevated in mice treated with AR9281 compared to the vehicle group. In addition, treatment with AR9281 significantly reduced fasting blood glucose levels in diabetic db/db mice.

It is reported that in the mesenteric arteries isolated from the wild-type control db +/? mice with pre-constricted tension as 100%, application of acetylcholine can reduce the tension by > 90%, a normal endothelium-dependent vasorelaxation response (Pannirselvam et al., 2002). However, in the mesenteric arteries isolated from vehicle-treated db/db mice (Fig. 1, top), the maximal vasorelaxation response to acetylcholine was greatly diminished ($E_{max}=36\pm6\%$), while the endothelial independent vasorelaxation to SNP remained intact ($E_{max}=93\pm2\%$). In the db/db mice treated with AR9281 for eight weeks, vasorelaxation to acetylcholine in the mesenteric artery was significantly enhanced ($E_{max}=58\pm6\%$) while the response to SNP was not affected ($94\pm2\%$). In comparison to responses in vehicle-treated animals, AR9281 did not affect the sensitivity of the mesenteric vasorelaxation response to acetylcholine ($pD_2=6.9\pm0.4$ vs. 6.4 ± 0.3, respectively) and SNP (pD_2: 7.6 ± 0.1 vs. 7.6 ± 0.1, respectively). In the presence of L-NAME and indomethacin, AR9281-induced enhancement of vasorelaxation to acetylcholine in the mesenteric arteries was maintained with a significantly greater sensitivity compared to that of the vehicle treated group ($pD_2=7.1\pm0.1$ vs. 5.6 ± 0.1, $p<0.05$, respectively).

In the aorta (Fig. 1, bottom), the vasorelaxation to acetylcholine, but not SNP was only slightly, but not significantly enhanced by AR9281. Incubation of the aorta with L-NAME and indomethacin completely abolished the responses to acetylcholine in both the vehicle and AR9281 groups.

Table 1
Blood s-EH activity, plasma oxylipids and fasting glucose in db/db mice treated with vehicle or AR9281.

	Vehicle (n = 10)	AR9281 (n = 10)	P values*
DHET (nM/min)	320 ± 57	8 ± 3	<0.001
EpOME/DiHOME ratio	0.11 ± 0.04	7.00 ± 0.81	<0.001
Fasting Glucose (mg/dL)	516 ± 22	377 ± 40	<0.01

* P value was obtained using Student's *t*-test for the statistical comparison between the vehicle and AR9281 groups.

3.2. AR9281 restored endothelium-dependent vasorelaxation in both the mesenteric artery and aorta in high fat and fructose diet-induced obese mice

HFF diet feeding not only increased body weight, but also elevated fasting glucose levels compared to those of chow fed mice (Table 2). In addition, whole blood s-EH activity was also significantly increased in DIO mice. Treatment of DIO mice with AR9281, which significantly inhibited whole blood s-EH activity and doubled the plasma EpOME/DiHOME ratio measured 6–10 h after the last dose reduced body weight and lowered fasting blood glucose (Table 2).

In the mesenteric artery, acetylcholine-induced vasorelaxation was significantly attenuated in DIO ($E_{max}=25\pm9\%$) compared to that of chow fed mice ($E_{max}=80\pm8\%$), $p<0.05$ (Fig. 2 top left). Chronic treatment of DIO mice with AR9281 for four weeks restored acetylcholine response to a level ($E_{max}=74\pm6\%$) close to that of chow-fed controls. There were no differences in vasorelaxation to SNP among the groups of chow fed control mice ($E_{max}=96\pm1\%$ and $pD_2=7.7\pm0.1$) and DIO mice treated with vehicle ($E_{max}=94\pm1\%$, and $pD_2=7.9\pm0.1$) or AR9281 ($E_{max}=97\pm2\%$ and $pD_2=7.8\pm0.1$) (Fig. 2. top right).

In the aorta, acetylcholine-induced vasorelaxation was also significantly reduced in DIO mice compared to that of chow-fed controls ($E_{max}=75\pm3$ vs. $89\pm2\%$, $p<0.05$). Treatment with AR9281 restored acetylcholine response in DIO mice ($E_{max}=89\pm3\%$) to the level of chow-fed controls (Fig. 2 bottom left). There was no significant difference in the vasorelaxation to SNP for both E_{max} (95 ± 2 vs. 96 ± 1 vs. $97\pm1\%$) and pD_2 (7.7 ± 0.1 vs. 7.9 ± 0.1 vs. 7.8 ± 0.1) among three treatment groups (Fig. 2 bottom right).

3.3. AR9281 and AR9276 restored aortic endothelium-dependent relaxation in rats chronically infused with angiotensin II

In rats chronically infused with angiotensin II for 4 weeks, vasorelaxation to acetylcholine was diminished in the aorta pre-constricted by phenylephrine compared to control rats without angiotensin II treatment (Fig. 3, top left). Concomitant treatment with AR9281 enhanced aortic relaxation to acetylcholine in angiotensin II-treated rats (Fig. 3, bottom left). The concentration–response curves showed that both AR9281 and AR9276 significantly enhanced acetylcholine-induced relaxation in the aorta from angiotensin II treated rats to the level of naive rats without treatment of angiotensin II (Fig. 3, right). There is no difference in vasorelaxatory sensitivity to acetylcholine among the four groups of normal rats without any treatment (naïve) and angiotensin II-infused rats treated with vehicle, AR9281 or AR9276 ($pD_2=7.3\pm0.2$ vs. 7.5 ± 0.1 vs. 7.6 ± 0.1 vs. 7.4 ± 0.1, respectively).

4. Discussion

These results confirm and extend previously published reports of endothelial dysfunction in animal models of diabetes, obesity and hypertension (De Ciuceis et al., 2007; Ding et al., 2005; Eichhorn et al., 2009; Noronha et al., 2005; Pannirselvam et al., 2003; Wattanapitayakul et al., 2000). Importantly, these data demonstrate that inhibition of s-EH by the novel s-EH inhibitors AR9281 and or AR9276 reversed endothelial dysfunction in three animal models of cardiometabolic

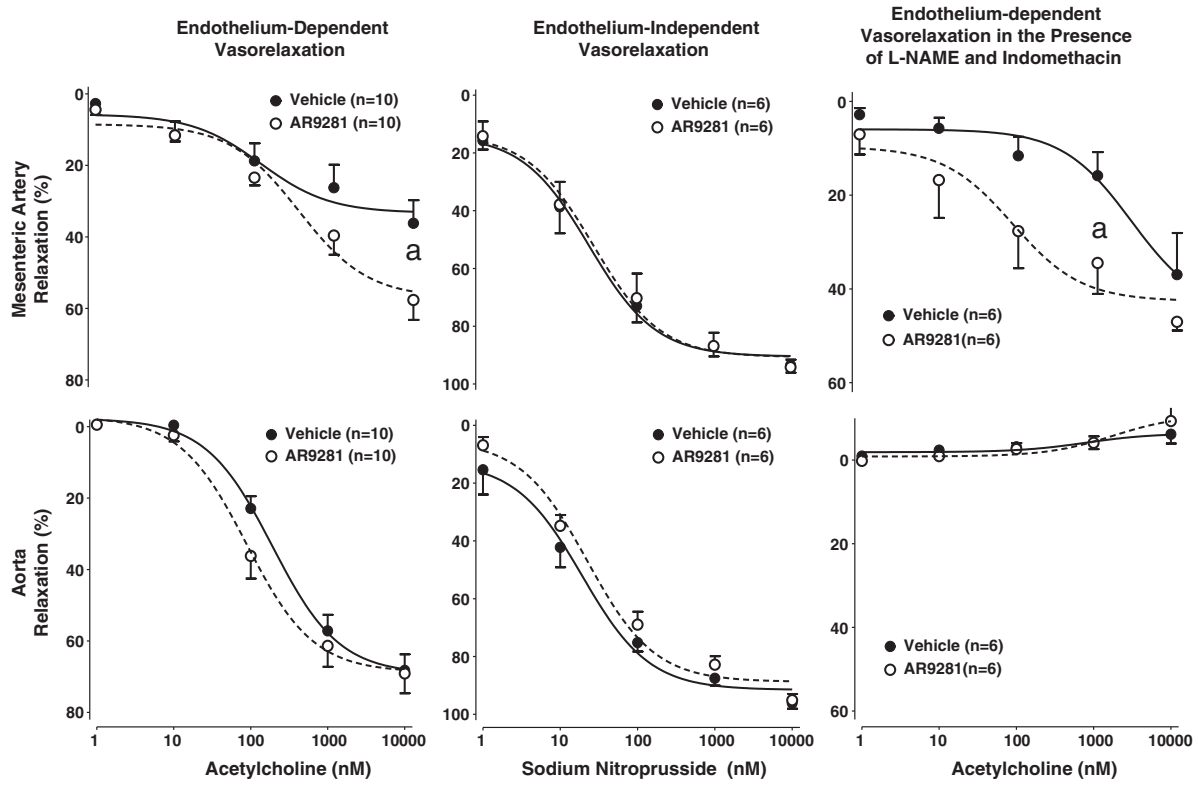

Fig. 1. AR9281 improved endothelial function in the mesenteric artery but not in the aorta, which was independent of the presence of nitric oxide synthase or cyclooxygenase. Concentration–response curves to acetylcholine (left), sodium nitroprusside (middle) and acetylcholine in the presence of L-NAME and indomethacin (right) in the isolated mesenteric arterial branch (top) and aorta (bottom) from diabetic db/db mice treated with vehicle or AR9281 for eight weeks. a $P<0.05$ AR9281 vs. vehicle group.

disease, namely diabetic db/db and DIO mice, and angiotensin II-induced hypertensive rats.

Endothelial dysfunction, measured by impaired endothelial dependent vasorelaxation, or blunted vascular response to endogenous vasodilators is a potential contributor to the pathogenesis of cardiometabolic disease, including diabetes mellitus, obesity and hypertension. Impaired blood perfusion could reduce efficiency of glucose delivery to tissues (Hadi and Suwaidi, 2007; Hartge et al., 2006). Indeed, endothelial dysfunction in small resistance arteries supplying blood perfusion to skeletal muscle has been shown to contribute to insulin resistance (Frisbee, 2001; Frisbee and Stepp, 2001). In addition, hyperglycemia may elicit endothelial dysfunction by elevation of superoxide production and oxidative stress (Rajapakse et al., 2009; Wadham et al., 2007). The present data demonstrate that AR9281 treatment improved glucose homeostasis and ameliorated endothelial dysfunction in diabetic db/db and DIO mice. Although these observations provide evidence for a reciprocal interaction between diabetes mellitus and endothelial dysfunction (Nathanson and Nystrom, 2009), they do not clarify the cause and effect relationship between the two.

Table 2
Body weight, fasting glucose, whole blood s-EH activity measured by the rate of DHET production, and oxylipids in chow or high fat and fructose (HFF) fed mice treated with vehicle or AR9281.

	Chow + Vehicle (n = 10)	HFF + Vehicle (n = 10)	HFF + AR9281 (n = 10)
Body weight (g)	31.2 ± 0.9	38.8 ± 1.0 [a]	35.6 ± 1.4 [b]
Fasting glucose (mg/dl)	144 ± 3	179 ± 4 [a]	145 ± 5 [b]
DHET (nM/min)	49 ± 9	92 ± 11 [a]	43 ± 8 [b]
EpOME/DiHOME ratio	3.3 ± 1.3	6.2 ± 1.5	13.4 ± 3.3

a, $P<0.05$ vs. chow + vehicle. b, $P<0.05$ vs. HFF + vehicle.

Accumulating evidence suggests that EETs play a role in modulation of vascular function in the development of insulin resistance and diabetes mellitus. Decreased CYP epoxygenase and increased s-EH expression were observed in obese Wistar Kyoto (Watkins and Mangels, 1987) and Zucker fatty rats (Zhao et al., 2005). In obese Zucker rats, fenofibrate restored endothelial function by increasing CYP epoxygenase expression and EET synthesis (Zhao et al., 2006). In accord with this study, insulin-resistant rats showed diminished EDHF-mediated vasodilatation and hypertension, and induction of CYP with phenobarbital restored endothelial function and normalized blood pressure in this model (Katakam et al., 2000). Thus, reduced EET availability and the consequent endothelial dysfunction might contribute to the pathogenesis of cardiometabolic syndrome and diabetes. The present data support this concept by showing that s-EH activity is elevated in DIO mice; and that s-EH inhibition by AR9281 treatment attenuates endothelial dysfunction and ameliorates hyperglycemia in both diabetic db/db and DIO mice.

NO and prostacyclin are important endothelial derived mediators implicated in the regulation of vascular tone (Esper et al., 2006). The present data showed that in the presence of L-NAME and indomethacin, which blocked NO- and prostacyclin-mediated vasorelaxation, enhancement of vasorelaxation by AR9281 in db/db mice is maintained in the mesenteric artery, thereby excluding a role for NO and prostacyclin in mediating the effects of AR9281 in these vessels. Interestingly, in the absence of L-NAME and indomethacin, AR9281 only enhanced the magnitude of response, but not the sensitivity of the vasorelaxation response to acetylcholine. In contrast, in the presence of L-NAME and indomethacin, the sensitivity of vasorelaxation response to acetylcholine was also increased in mice treated with AR9281, indicating that the absence of NO and prostacyclin may sensitize the vascular response to EET/EDHF. The present data also demonstrated that acetylcholine-induced vasorelaxation in the aorta, but not in the mesenteric artery, was completely blocked by NOS and

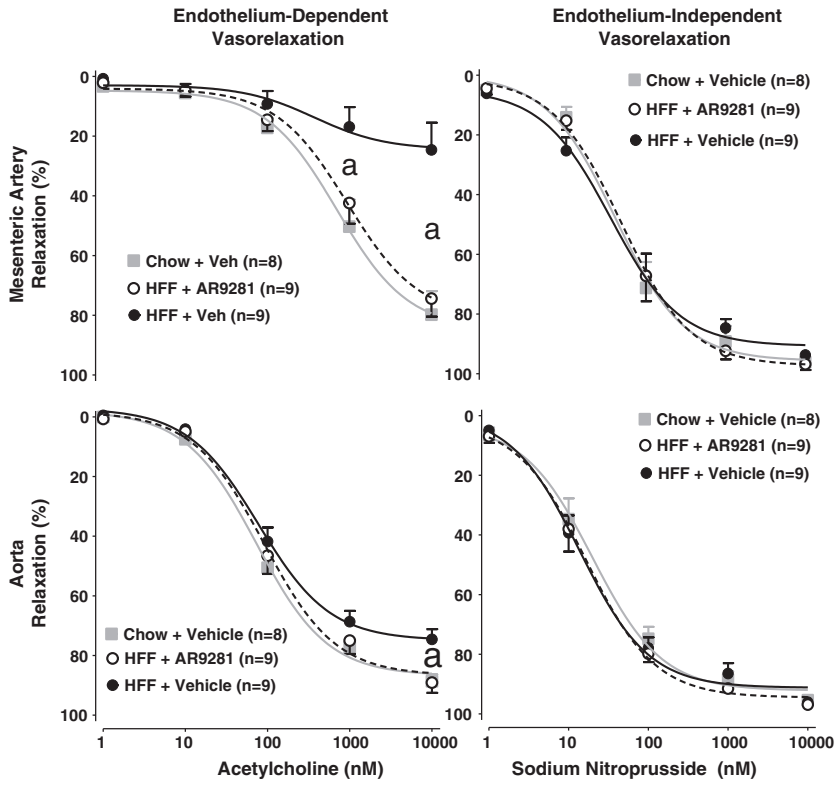

Fig. 2. Concentration–response curves to acetylcholine (left) and sodium nitroprusside (right) in isolated mesenteric arterial branches (top) aorta (bottom) from chow or high fat and fructose (HFF) diet fed mice treated with vehicle or AR9281. a $P<0.05$ AR9281 vs. vehicle group.

cyclooxygenase inhibition in vessels from both vehicle and AR9281 treated mice, and that AR9281 ameliorated endothelial dysfunction only in the mesenteric artery but not in the aorta in db/db mice. These findings reinforce the concept that involvement of EETs as components of EDHF is more prominent in small resistance arteries, and that elevating EETs by AR9281-mediated inhibition of s-EH prevented vascular complications in this diabetic mouse model via pathways independent of NO and prostacyclin. Consistent with this observation, a recent study reported that treatment with another s-EH inhibitor, 12-(3-adamantan-1-yl-ureido) dodecanoic acid (AUDA) decreased renal vascular resistance, increased renal blood flow, improved abnormal renal hemodynamics, and reduced hypertension in obese hypertensive rats by stabilizing EETs (Huang et al., 2007).

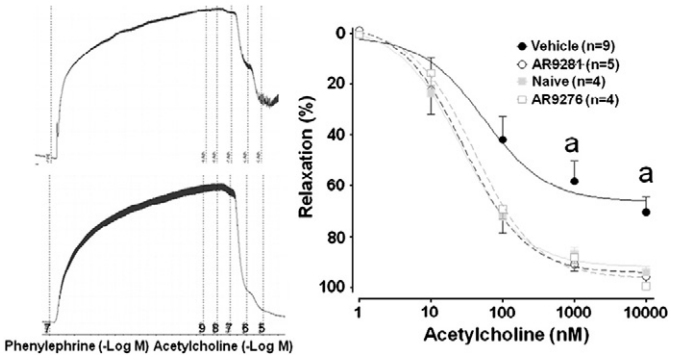

Fig. 3. AR9281 and AR9276 attenuated endothelial dysfunction in rats chronically infused with angiotensin II. Left: Original traces of acetylcholine-induced relaxation of phenylephrine pre-contracted aorta isolated from a vehicle- (top) and AR9281 (bottom)-treated rat. Right: Concentration–response curves to acetylcholine in the isolated aorta from angiotensin II-infused rats treated with vehicle or AR9281 or AR9276 for two weeks, as well as normal naïve rats. [a] $P<0.05$ AR9281 vs. vehicle group.

Several s-EH inhibitors have been shown to decrease blood pressure in animal models of hypertension (Imig, 2006), including NCND (N-cyclohexyl-N-dodecyl urea) (Imig et al., 2002), AUDA (Jung et al., 2005) and ADU (N-adamantyl-N′-dodecylurea) (Hercule et al., 2009) in angiotensin II-induced hypertension in rats and mice. It is well-established that endothelial dysfunction is an important contributor to angiotensin II-induced hypertension in these animal models (Sarr et al., 2006). The current data showed that s-EH inhibition by AR9281 attenuated angiotensin II-induced endothelial dysfunction in rat aorta. This result is consistent with the finding by Loch et al. (2007) who showed that ADU restored the endothelial function of the thoracic aorta in DOCA-salt hypertensive rats. In the aorta, acetylcholine-induced vasorelaxation is mainly mediated by NO. An increase in vascular superoxide and the consequent decrease in NO bioavailability are important mechanisms of angiotensin II-induced endothelial dysfunction in the aorta (Oelze et al., 2006; Rajagopalan et al., 1996). EDHF could restore endothelial function by increasing NO levels via quenching excess superoxide (Kaw and Hecker, 1999). It has been reported that EETs may activate endothelial NO synthase (eNOS) and NO release (Hercule et al., 2009; Wang et al., 2003). Consequently, restoring aortic NO function by increasing EET levels via s-EH inhibition could be one of the mechanisms whereby AR9281 restored endothelial function in angiotensin II-induced hypertensive rats. This interpretation was supported by earlier observations that CYP-derived EDHF is involved in maintaining blood flow in human conduit arteries (Bellien et al., 2006) and s-EH inhibition by CUDA (N-cyclohexyl-N′-dodecanoic acid urea) increased EET levels in human aorta (Fang et al., 2004). In addition, angiotensin II has been shown to directly up-regulate s-EH expression and decrease EET levels (Ai et al., 2007; Imig et al., 2002). Thus, elevation of EET levels by inhibition of s-EH and, augmentation of EET-dependent vascular functions could be a mechanism whereby s-EH inhibitors prevent the detrimental effects of angiotensin II on endothelial function in the rat aorta.

The concentration of AR9281 in the drinking water was 5 fold higher in db/db mice compared to that in angiotensin II-induced hypertensive rats. Since daily water consumption in diabetic db/db mice was doubled compared to that in rats, the calculated daily dose of AR9281 was ~10 fold greater in db/db mice. In both cases, the blood s-EH activity was completely inhibited, which should contribute to the beneficial effects on endothelium dysfunction in both models.

In addition, it has been reported that s-EH inhibitors have a prominent role in the kidney, e.g., inhibition of salt and water reabsorption, thus causing natriuresis and diuresis, which was attributed to the reported anti-hypertensive effects. However, in the present study, we did not observe obvious diuretic effects of AR9281 or AR9276. The fluid consumption between the vehicle control and AR9281 or AR9276 groups was not significantly different (data not shown). Thus, the natriuresis and dieresis may not be a predominant mechanism in the present studies.

In summary, the present data demonstrate that inhibition of s-EH by AR9281 restored endothelial function in three different animal models that reflect components of cardiometabolic syndrome. In small resistance arteries, the effects of AR9281 were mediated by a NO- and prostacyclin-independent mechanism suggesting involvement of an enhanced EDHF activity resulting from elevated EET levels. Along with recent reports of anti-atherogenic and lipid lowering properties of s-EH inhibitors in apolipoprotein E deficient mice (Ulu et al., 2008; Zhang et al., 2009), the present data suggest that inhibition of s-EH could be a promising target for the treatment of cardiometabolic disorders.

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Conflict(s) of Interest/Disclosure(s)

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