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MITOCHONDRIAL ALDEHYDE DEHYDROGENASE PREVENTS ROS-INDUCED VASCULAR CONTRACTION IN ANGIOTENSIN II HYPERTENSIVE MICE

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Abstract

Background—Mitochondrial aldehyde dehydrogenase (ALDH2) is an enzyme that detoxifies aldehydes to carboxylic acids. ALDH2 deficiency is known to increase oxidative stress, which is the imbalance between reactive oxygen species (ROS) generation and antioxidant defense activity. Increased ROS contribute to vascular dysfunction and structural remodeling in hypertension. We hypothesized that ALDH2 plays a protective role to reduce vascular contraction in angiotensin II (AngII) hypertensive mice.

Methods and Results—Endothelium-denuded aortic rings from C57BL6 mice, treated with AngII (3.6 µg/kg/min, 14 days), were used to measure isometric force development. Rings treated with daidzin (10 µmol/L), an ALDH2 inhibitor, potentiated contractile responses to phenylephrine (PE) in AngII mice. Tempol (1 mmol/L) and catalase (600U/ml) attenuated the augmented contractile effect of daidzin. In normotensive mice, contraction to PE in the presence of the daidzin was not different from control, untreated values. AngII aortic rings transfected with ALDH2 recombinant protein decreased contractile responses to PE compared with control.

Conclusions—These data suggest that ALDH2 reduces vascular contraction in AngII hypertensive mice. Since tempol and catalase blocked the contractile response of the ALDH2 inhibitor, ROS generation by AngII may be decreased by ALDH2, thereby preventing ROS-induced contraction.

Keywords

Hypertension; ROS; ALDH2; Vascular contraction

Introduction

Oxidative stress occurs when there is an increase in reactive oxygen species (ROS) generation compared to the normal antioxidant defense system and is defined as a disruption of redox signaling and control (1). ROS are small molecules derived from molecular oxygen, and their free radicals play a major role in signal transduction, including mitogen-activated protein (MAP) kinase pathways (2;3). ROS increase vascular tone and can

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participate in arterial contraction. Furthermore, increased vascular ROS contributes to vascular dysfunction and structural remodeling, establishing its role in increasing blood pressure (4–7). The effect of ROS on blood pressure has been shown in many hypertensive models including angiotensin II (AngII)-induced models (8–12).

AngII contributes to many physiological responses including vasoconstriction, aldosterone release, water and sodium retention, and elevated sympathetic activity. Additionally, AngII causes vascular smooth muscle cell growth, modulates myocardial hypertrophy, and modulates ventricular remodeling (13–17). Importantly, many studies have reported that AngII produces superoxide and that in AngII-induced hypertension, ROS generation is increased via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and/or degradation of endothelium-derived nitric oxide (NO) (18–20). Thus, elevated oxidative stress through increased ROS is an important factor in AngII-induced hypertension.

The mitochondrial aldehyde dehydrogenase (ALDH2) has three enzymatic activities. First, the dehydrogenase activity converts aldehyde into carboxylic acid. Second, the esterase activity converts carboxylic acid ester to the free carboxylic acid and alcohol. Third, the reductase activity was recently described as a bioactivation enzyme for organic nitrates such as nitroglycerin (glyceryl trinitrate, GTN) (21). Furthermore, ALDH2 was shown to reduce ROS formation related to toxic aldehydes, which cause lipid peroxidation. In cultured cells as well as in mice, ALDH2 deficiency increases oxidative stress, suggesting that this enzyme acts as an important antioxidant (22;23). In this study, we examined if ALDH2 plays a role as an antioxidant in AngII-induced hypertensive mice aorta. To investigate this hypothesis, we tested vascular reactivity using AngII mice aorta in the presence or absence of an ALDH2 inhibitor.

Methods

Materials

Phenylephrine (PE), acetylcholine, daidzin, 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy (tempol), catalase, and *N*-nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma-Aldrich (St. Louis, MO). AngII was obtained from Phoenix Pharmaceuticals, Inc. (Burlingame, CA). ALDH2 recombinant protein was purchased from Novus biologicals (Littleton, CO).

Animals

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) in 12–14 weeks of age were used in this study. To induce the AngII hypertensive mice model, osmotic minipumps (Durect Corporation, Cupertino, CA) with AngII (3.6 µg/kg/min) were implanted subcutaneously into the dorsum for 14 days. All procedures were approved by the institutional animal care committee.

Isolation of aortic rings and vascular functional studies

After mice were euthanized, thoracic aorta were excised, cleaned of fat tissue and cut into 2 mm length rings in an ice-cold physiological salt solution consisting of the following: 130 mmol/L NaCl, 4.7 mmol/L KCl, 1.18 mmol/L KH₂PO₄, 1.18 mmol/L MgSO₄·7H₂O, 1.56 mmol/L CaCl₂·2H₂O, 14.9 mmol/L NaHCO₃, 5.6 mmol/L glucose, and 0.03 mmol/L EDTA. Aortic rings were mounted in a myograph (Danish Myo Technology A/S, Aarhus, Denmark) containing warmed (37°C), oxygenated (95% O₂/5% CO₂) physiological salt solution. The preparations were equilibrated for 1 hour under a passive tension of 5 mN. After equilibration, arterial integrity was assessed by a depolarizing concentration of 120

mmol/L KCl, then washed, and finally stimulated with PE (0.1 $\mu\text{mol/L}$) followed by relaxation with acetylcholine (1 $\mu\text{mol/L}$), which was used as an evidence of an intact endothelium. Some aortic rings were incubated with L-NAME (100 $\mu\text{mol/L}$, 40 minutes), daidzin (10 $\mu\text{mol/L}$, 20 minutes), tempol (1 mmol/L, 20 minutes), or catalase (600 U/ml, 30 minutes). Cumulative concentration-response curves to PE, using 10^{-9} to 10^{-5} mol/L, were performed.

Recombinant protein delivery

To deliver recombinant protein into the aortic rings, chariot protein delivery reagent (Active Motif, Carlsbad, CA) was used according to the manufacturer's instruction as described previously (24). Empty chariot reagent was used as control and chariot-ALDH2 complex was used for ALDH2 overexpression. In brief, 6 μl of chariot reagent in 100 μl of 40% dimethyl sulfoxide (DMSO) were mixed with 3 μg of protein in 100 μl of phosphate buffered saline (PBS) and incubated at room temperature for 30 minutes. The aortas were transferred to 400 μl of Dulbecco's modified Eagle's medium (DMEM) in 24-well cell culture plate and 200 μl of the protein/chariot complexes were added. Aortas were incubated for 1 hour at 37 °C CO₂ incubator, followed by addition of DMEM (750 μl) and further incubated for 2 hours at 37 °C CO₂ incubator. Subsequently, the aortic rings were mounted in the myograph, and functional studies were performed.

Dihydroethidium (DHE) staining

In situ superoxide generation was evaluated in vascular cryosections with the superoxide-sensitive fluorescent dye DHE (Invitrogen, Eugene, OR). Briefly, after incubation with vehicle (control) or daidzin (10 $\mu\text{mol/L}$) for 1 hour, aortas were frozen in optimal cutting temperature, and cryosections (7 μm) were obtained (LEICA CM3050S). After washing with PBS, cryosections were incubated with vehicle or daidzin for 30 min and then incubated with DHE (10 $\mu\text{mol/L}$) in PBS for 30 min at 37 °C. Fluorescent images were obtained using fluorescence microscope (ZEISS; Carl Zeiss, Thornwood, NY) and analyzed by ImageJ program.

Western blot analysis

Proteins (40 μg) extracted from aorta in lysis buffer (20 mmol/L Tris-HCl, pH 7.4, 5 mmol/L Na₂P₂O₇, 100 mmol/L NaF, 2 mmol/L Na₃VO₄, 1% NP-40, protease inhibitor cocktail, and 1 mmol/L PMSF) were separated by electrophoresis on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were blocked with 5% skim milk in Tris-buffered saline solution with Tween-20 for 1 h at room-temperature. Membranes were then incubated with ALDH2 (Santa Cruz Biotechnology, Santa Cruz, CA) or β -actin (Sigma-Aldrich) primary antibody overnight at 4°C. After incubation with secondary antibody, signals were exposed with chemiluminescence, visualized by autoradiography, and quantified densitometrically.

Statistical analysis

Values are mean $\pm$ standard error of the mean (SEM), and 'n' represents the number of animals used in the experiments. Contractions were recorded as changes in the displacement (mN) from baseline and were expressed as percent change from 120 mmol/L KCl-contraction values. Concentration-response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 4.0; GraphPad Software, San Diego, CA), and 2 pharmacological parameters were obtained: the maximal effect generated by the agonist (or E_{max}) and EC₅₀. Statistical differences were calculated by Student's *t*-test or one-way ANOVA. A *P* value less than 0.05 was considered to be statistically significant.

Results

The effects of daidzin on vascular function

To compare vascular reactivity in normotensive and hypertensive mice in the presence of daidzin, an ALDH2 selective inhibitor (23;25), we first tested aortic contraction to the α 1-adrenergic agonist, PE, using sham mice (14 days, post-surgery). To determine the function of vascular smooth muscle, we used both endothelium-denuded vessels and endothelium-intact vessels treated with 10^{-4} mol/L L-NAME (nitric oxide synthase inhibitor). No differences were observed in vascular reactivity to PE in the presence and absence of daidzin (Figure 1), suggesting that ALDH2 has no effect on contraction in normotensive aortic rings.

After 14 days of AngII treatment, PE-mediated contractile activity was evaluated in aortas from hypertensive mice. Contractile responses to PE were increased in the presence of daidzin compared with vehicle groups in AngII mice aorta. These results were similar in the both aortic rings from endothelium-denuded and endothelium-intact aortic segments with L-NAME (Figure 2), indicating that ALDH2 plays an important role in vascular contraction in AngII hypertensive mice aorta. Importantly, the augmented contractile effect of daidzin appeared in only AngII mice aorta and not in the normotensive aorta, suggesting that ALDH2 is related to AngII-induced changes in vascular function.

ALDH2 in AngII mice aorta

As an alternative approach to evaluate the role of ALDH2 in vascular contraction, ALDH2 recombinant protein was intracellularly delivered by the chariot reagent system. Figure 3 shows that transfection with ALDH2 resulted in decreased vascular contraction to PE, suggesting that ALDH2 plays an important role to ameliorate reactivity in AngII mice aorta.

Inhibition of ROS and ALDH2

Considering that ALDH2 leads to a decreased contractile response via antioxidant activity, we determined whether contractile activity in the presence of daidzin from AngII aortic rings is related to changes in ROS. Tempol is a superoxide dismutase mimetic and is used as a superoxide anion scavenger. Catalase mediates the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. These two compounds play a role in decreasing ROS-induced vascular contraction (7). As shown in Figure 4, tempol and catalase attenuated the augmented vascular contraction induced by daidzin. To further examine aortic superoxide generation, DHE staining was performed and daidzin increased superoxide levels in AngII aorta cryosections (Figure 5), indicating that daidzin induces ROS-related vascular contraction in AngII mice aorta.

To consider whether AngII-induced hypertension is associated to ALDH2 expression, ALDH2 protein level was determined in sham and AngII mice aorta. As shown in Figure 6, ALDH2 was decreased in AngII compared to sham.

Discussion

Recent studies have shown that ALDH2 is important in the catalytic reduction of GTN, generating nitrite and 1,2-glyceryl dinitrate (21;26;27). GTN has been used to treat angina pectoris, myocardial infarction, and heart failure. However, long-term treatment of mice with GTN causes nitrate tolerance and vasodilation to GTN is impaired compared to normal mice. These studies have demonstrated that vascular tolerance to GTN caused by impaired GTN bioactivation is due to ALDH2 inactivation (27). Accordingly, ALDH2 is related to vascular reactivity in the case of the nitrate tolerance.

In this study, we investigated a role of ALDH2 in vascular contraction, exploring other activities of this enzyme. We tested the hypothesis that ALDH2 has protective role in vascular smooth muscle contraction from AngII-induced hypertensive mice. Our results showed that inhibition of ALDH2 induced an increase in aortic contraction from only AngII mice and not sham control group (Figure 1 and 2). AngII is well known as a vasoconstrictor, which contributes to elevation in blood pressure and produces superoxide via membrane NADPH oxidase activation (8). Furthermore, AngII induces hypertension via several different signaling pathways, including G-protein mediated activation of phospholipase C, which elevates intracellular calcium concentration, and activates diacylglycerol signaling, protein kinase C, and MAP kinase pathway (28;29). In addition to these signaling pathway, AngII induces mitochondrial dysfunction (30). Since several protein signaling cascades are activated by AngII compared to normal condition and ALDH2 is a mitochondrial protein, the effect of ALDH2 in only AngII mice aorta may be related to some of these signaling pathways and mitochondrial function.

To further determine mechanisms associated with AngII and ALDH2 on vascular function, this study focused on the relationship between ROS generation by AngII and ALDH2. Previously it has been demonstrated that ALDH2 deficiency increases oxidative stress in cell culture and mouse models (22;23). There also appears to be a possible indirect mechanism, which is through oxidative stress for lipid peroxidation. Inactivated ALDH2 contributes to accumulated aldehyde and they can induce lipid peroxidation. Thus, ALDH2 deficiency may increase oxidative stress via lipid peroxidation (31).

Oxidative stress occurs when the generation of ROS is greater than elimination through antioxidant mechanisms and is linked to many diseases. ROS have important roles in vascular function. For example, Jin et al. (7) showed that ROS activate RhoA, which increases Rho kinase activity, and results in contraction in rat aorta. ROS generated by xanthine and xanthine oxidase induce smooth muscle contraction and are inactivated by antioxidants, such as tempol and catalase. Antioxidants include several enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. As shown in Figure 4, tempol, a superoxide dismutase mimetic, and catalase reduced vascular contractile responses. These two antioxidants abolished the augmented contraction by daidzin, suggesting that daidzin increases ROS generation via ALDH2 inactivation in AngII mice. These data indicate that in AngII mice, increased ROS contribute to elevated vascular contraction, which can be inactivated by ALDH2. Therefore, we conclude that ALDH2 plays a role in an antioxidant to improve vascular reactivity in AngII mice aorta.

Further studies will be necessary to clarify the mechanism of ROS inhibition by ALDH2 in the vasculature and whether ALDH2 contributes to signaling related to ROS, such as MAP kinase or Rho kinase. Additionally, the present study did not demonstrate whether ALDH2 affects blood pressure and development of hypertension, thus the ALDH2 mechanism in hypertension remains unclear.

In summary, ROS in hypertension and vascular dysfunction is considered as a therapeutic target and ALDH2 may be considered a novel antioxidant in vascular function. For the first time, we have shown that ALDH2 inhibition is related to ROS-induced vascular contraction in AngII hypertensive models. Because our data strongly support the protective role of ALDH2, this enzyme may be a target for treatment of hypertension.

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Reference List

1. Jones DP. Redefining oxidative stress. *Antioxid Redox Signal*. 2006 Sep; 8(9–10):1865–79. [PubMed: 16987039]
2. Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, et al. Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. *Recent Prog Horm Res*. 2001; 56:127–55. [PubMed: 11237210]
3. Nishida E, Gotoh Y. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci*. 1993 Apr; 18(4):128–31. [PubMed: 8388132]
4. uch-Schwelk W, Katusic ZS, Vanhoutte PM. Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. *Hypertension*. 1989 Jun; 13(6 Pt 2):859–64. [PubMed: 2567706]
5. Yang ZW, Zheng T, Wang J, Zhang A, Altura BT, Altura BM. Hydrogen peroxide induces contraction and raises $[Ca^{2+}]_i$ in canine cerebral arterial smooth muscle: participation of cellular signaling pathways. *Naunyn Schmiedebergs Arch Pharmacol*. 1999 Dec; 360(6):646–53. [PubMed: 10619181]
6. Rodriguez-Martinez MA, Garcia-Cohen EC, Baena AB, Gonzalez R, Salaices M, Marin J. Contractile responses elicited by hydrogen peroxide in aorta from normotensive and hypertensive rats. Endothelial modulation and mechanism involved. *Br J Pharmacol*. 1998 Nov; 125(6):1329–35. [PubMed: 9863664]
7. Jin L, Ying Z, Webb RC. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. *Am J Physiol Heart Circ Physiol*. 2004 Oct; 287(4):H1495–H1500. [PubMed: 15371261]
8. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griending KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest*. 1996 Apr 15; 97(8):1916–23. [PubMed: 8621776]
9. Romero JC, Reckelhoff JF. State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension. *Hypertension*. 1999 Oct; 34(4 Pt 2):943–9. [PubMed: 10523389]
10. Schnackenberg CG, Welch WJ, Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. *Hypertension*. 1998 Jul; 32(1):59–64. [PubMed: 9674638]
11. Wilson SK. Role of oxygen-derived free radicals in acute angiotensin II--induced hypertensive vascular disease in the rat. *Circ Res*. 1990 Mar; 66(3):722–34. [PubMed: 2306804]
12. Wu R, Millette E, Wu L, de CJ. Enhanced superoxide anion formation in vascular tissues from spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats. *J Hypertens*. 2001 Apr; 19(4):741–8. [PubMed: 11330877]
13. Berk BC, Vekshtein V, Gordon HM, Tsuda T. Angiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension*. 1989 Apr; 13(4):305–14. [PubMed: 2466788]
14. Fernandez-Alfonso MS, Ganten D, Paul M. Mechanisms of cardiac growth. The role of the renin-angiotensin system. *Basic Res Cardiol*. 1992; 87(Suppl 2):173–81. [PubMed: 1299208]
15. Lee MA, Bohm M, Paul M, Ganten D. Tissue renin-angiotensin systems. Their role in cardiovascular disease. *Circulation*. 1993 May; 87(5 Suppl):IV7–13. [PubMed: 8485836]
16. Paul M, Ganten D. The molecular basis of cardiovascular hypertrophy: the role of the renin-angiotensin system. *J Cardiovasc Pharmacol*. 1992; 19(Suppl 5):S51–S58. [PubMed: 1381795]
17. Everett AD, Tufro-McReddie A, Fisher A, Gomez RA. Angiotensin receptor regulates cardiac hypertrophy and transforming growth factor-beta 1 expression. *Hypertension*. 1994 May; 23(5):587–92. [PubMed: 8175166]
18. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation*. 1997 Feb 4; 95(3):588–93. [PubMed: 9024144]

19. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, et al. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension*. 2002 Oct; 40(4):511–5. [PubMed: 12364355]
20. Wang HD, Xu S, Johns DG, Du Y, Quinn MT, Cayatte AJ, et al. Role of NADPH oxidase in the vascular hypertrophic and oxidative stress response to angiotensin II in mice. *Circ Res*. 2001 May 11; 88(9):947–53. [PubMed: 11349005]
21. Chen Z, Zhang J, Stamler JS. Identification of the enzymatic mechanism of nitroglycerin bioactivation. *Proc Natl Acad Sci U S A*. 2002 Jun 11; 99(12):8306–11. [PubMed: 12048254]
22. Szocs K, Lassegue B, Wenzel P, Wendt M, Daiber A, Oelze M, et al. Increased superoxide production in nitrate tolerance is associated with NAD(P)H oxidase and aldehyde dehydrogenase 2 downregulation. *J Mol Cell Cardiol*. 2007 Jun; 42(6):1111–8. [PubMed: 17493633]
23. Wenzel P, Muller J, Zurmeyer S, Schuhmacher S, Schulz E, Oelze M, et al. ALDH-2 deficiency increases cardiovascular oxidative stress--evidence for indirect antioxidative properties. *Biochem Biophys Res Commun*. 2008 Feb 29; 367(1):137–43. [PubMed: 18157936]
24. Giachini FR, Chiao CW, Carneiro FS, Lima VV, Carneiro ZN, Dorrance AM, et al. Increased activation of stromal interaction molecule-1/Orai-1 in aorta from hypertensive rats: a novel insight into vascular dysfunction. *Hypertension*. 2009 Feb; 53(2):409–16. [PubMed: 19075091]
25. Keung WM, Vallee BL. Daidzin: a potent, selective inhibitor of human mitochondrial aldehyde dehydrogenase. *Proc Natl Acad Sci U S A*. 1993 Feb 15; 90(4):1247–51. [PubMed: 8433985]
26. DiFabio J, Ji Y, Vasilou V, Thatcher GR, Bennett BM. Role of mitochondrial aldehyde dehydrogenase in nitrate tolerance. *Mol Pharmacol*. 2003 Nov; 64(5):1109–16. [PubMed: 14573760]
27. Huellner MW, Schrepfer S, Weyand M, Weiner H, Wimplinger I, Eschenhagen T, et al. Inhibition of aldehyde dehydrogenase type 2 attenuates vasodilatory action of nitroglycerin in human veins. *FASEB J*. 2008 Jul; 22(7):2561–8. [PubMed: 18272654]
28. Yin G, Yan C, Berk BC. Angiotensin II signaling pathways mediated by tyrosine kinases. *Int J Biochem Cell Biol*. 2003 Jun; 35(6):780–3. [PubMed: 12676164]
29. Kaschina E, Unger T. Angiotensin AT1/AT2 receptors: regulation, signalling and function. *Blood Press*. 2003; 12(2):70–88. [PubMed: 12797627]
30. Doughan AK, Harrison DG, Dikalov SI. Molecular mechanisms of angiotensin II-mediated mitochondrial dysfunction: linking mitochondrial oxidative damage and vascular endothelial dysfunction. *Circ Res*. 2008 Feb 29; 102(4):488–96. [PubMed: 18096818]
31. Ohsawa I, Nishimaki K, Yasuda C, Kamino K, Ohta S. Deficiency in a mitochondrial aldehyde dehydrogenase increases vulnerability to oxidative stress in PC12 cells. *J Neurochem*. 2003 Mar; 84(5):1110–7. [PubMed: 12603834]

Figure 1.

Vascular contraction is not changed by daidzin (ALDH2 inhibitor) in normotensive mice aorta. Concentration-dependent contractile responses to PE (10⁻⁹ to 10⁻⁵ mol/L) were investigated after incubation with daidzin (10 μmol/L, 20 minutes). A: Endothelium-denuded aortic segments (n=4 to 5). B: Endothelium-intact aortic segments were used in the presence of L-NAME (10⁻⁴ mol/L) (n=7). Experimental values of contraction were calculated relative to the contractile response produced by 120 mmol/L KCl, which was taken as 100%. Sham represents normotensive mice. Vehicle is DMSO. Results are presented as mean ± SEM in each experimental group.

Figure 2.

PE-induced contractile response is increased in the presence of daidzin in AngII hypertensive mice aorta. After incubation with daidzin (10 μ mol/L or indicated concentration) for 20 minutes, concentration-response curves to PE were performed. A: Endothelium-denuded aortic segments (n=7 to 8). B: Endothelium-intact aortic segments were incubated with L-NAME (10⁻⁴ mol/L) for 20 minutes before daidzin treatment (n=7). C: Endothelium-denuded aortic segments in the presence or absence of indicated concentration of daidzin (n=3 to 5). AngII represents AngII-induced hypertensive mice as described in methods. Vehicle is DMSO. Results are presented as mean $\pm$ SEM in each experimental group. *, $P < 0.05$ vs. AngII-Vehicle.

Figure 3.

ALDH2 decreases vascular contraction to PE in AngII mice aorta. ALDH2 recombinant protein was delivered with the chariot reagent as described in methods. Control group was delivered with chariot reagents (n=8). Results are presented as mean $\pm$ SEM in each experimental group. *, $P < 0.05$ vs control.

Figure 4.

Tempol and catalase attenuate the augmented contractile response of daidzin in AngII mice aorta. A: Aortas were incubated with 1 mmol/L tempol for 40 minutes and/or 10 μmol/L daidzin for 20 minutes and investigated for vascular contraction to PE (n=6 to 9). B: Aortas were incubated with catalase (600 U/ml) for 30 minutes and/or 10 μmol/L daidzin for 20 minutes (n=6). Results are presented as mean ± SEM in each experimental group. *, $P<0.05$.

Figure 5. Superoxide levels in aortas are increased by daidzin treatment in AngII. A, B, and C: Representative fluorescent images (DHE staining) from control in AngII, daidzin in AngII, and control in sham, respectively. D: Bar graphs showing the fluorescence intensity of DHE dye. Bar represents means $\pm$ SEM in each experimental group. *, $P < 0.05$ ($n = 4$ to 6).

Figure 6. ALDH2 was reduced in AngII aorta compared to sham. A: Western blot analysis. B: Bar graphs showing the relative expression of proteins after normalization to β -actin expression. The sham group was set to one and bar represents means $\pm$ SEM in each experimental group. *, $P < 0.05$ vs. sham ($n = 6$).