Published in final edited form as:
Hypertension. 2010 October ; 56(4): 667–674. doi:10.1161/HYPERTENSIONAHA.110.154518.

CYTOCHROME P450 1B1 CONTRIBUTES TO ANGIOTENSIN II-INDUCED HYPERTENSION AND ASSOCIATED PATHOPHYSIOLOGY

Brett L. Jennings[1], **Seyhan Sahan-Firat**[1], **Anne M. Estes**[1], **Kanak Das**[1], **Nasreen Farjana**[1], **Xiao R. Fang**[1], **Frank J. Gonzalez**[2], and **Kafait U. Malik**[1]

[1]Department of Pharmacology, College of Medicine, 874 Union Avenue, University of Tennessee Health Science Center, Memphis, TN 38163

[2]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD 20892

Abstract

Hypertension is the leading cause of cardiovascular diseases, and angiotensin II is one of the major components of the mechanisms that contribute to the development of hypertension. However, the precise mechanisms for the development of hypertension are unknown. Our recent study that angiotensin II-induced vascular smooth muscle cell growth is dependent on cytochrome P450 1B1 led us to investigate its contribution to hypertension caused by this peptide. Angiotensin II was infused via miniosmotic pump into rats (150 ng/kg/min) or mice (1000 μg/kg/day) for 13 days resulting in increased blood pressure, increased cardiac and vascular hypertrophy, increased vascular reactivity to vasoconstrictor agents, increased reactive oxygen species production, and endothelial dysfunction in both species. The increase in blood pressure and associated pathophysiological changes were minimized by the cytochrome P450 1B1 inhibitor, 2,3′,4,5′-tetramethoxystilbene in both species and was markedly reduced in *Cyp1b1*$^{-/-}$ mice. These data suggest that cytochrome P450 1B1 contributes to angiotensin II-induced hypertension and associated pathophysiological changes. Moreover, 2,3′,4,5′-tetramethoxystilbene which prevents both cytochrome P450 1B1-dependent and independent components of angiotensin II-induced hypertension and inhibits associated pathophysiological changes could be clinically useful in the treatment of hypertension and associated cardiovascular and inflammatory diseases.

Keywords

angiotensin II; cytochrome P450 1B1; *Cyp1b1*$^{-/-}$ mice; blood pressure; cardiac and vascular hypertrophy; vascular reactivity; endothelial function

Corresponding author: Kafait U. Malik, D.Sc., Ph.D., Professor of Pharmacology, Department of Pharmacology, College of Medicine, 874 Union Avenue, University of Tennessee Health Science Center, Memphis, TN 38163 kmalik@uthsc.edu Ph: (901)-448-6075; Fax: (901)-448-7206.

Disclosures
None

Introduction

Angiotensin II (Ang II) is a major component of the mechanisms regulating cardiovascular homeostasis by maintaining vascular tone and salt and water balance (1). Ang II also activates cytosolic phospholipase A_2 and releases arachidonic acid (AA) from phospholipids (2). AA is metabolized by cyclooxygenase into prostaglandins and thromboxane A_2, by lipoxygenase into 5-, 12-, and 15-hydroxyeicosatetraenoic acids (HETEs), by cytochrome P450 (CYP4) ω-hydroxylase into 20-HETE, and by epoxygenase into epoxyeicosatrienoic acids (EETs) (3). Prostaglandins E_2 and I_2 and EETs contribute to antihypertensive mechanisms (4,5), whereas prostaglandin precursor PGH_2, and 20-HETE contribute to prohypertensive mechanisms (6-8). The balance between these anti- and prohypertensive eicosanoids, together with other vasoactive agents, determines blood pressure levels. Products of AA generated via lipoxygenase (12-HETE) or CYP4 (20-HETE) also promote vascular smooth muscle cell (VSMC) migration, proliferation, or hypertrophy by activating ERK1/2 and p38 MAPK (9-13) and contribute to the vasoconstrictor action of Ang II (14). Moreover, inhibitors of lipoxygenase and CYP4A minimize Ang II-dependent hypertension (6,15,16). Ang II and AA also stimulate production of reactive oxygen species (ROS) and activate c-JNK and p38 MAPK but not ERK1/2 in VSMCs (17-19) and Ang II-induced hypertension (20).

In addition to cyclooxygenase, lipoxygenase and CYP4A, other CYP enzymes can also metabolize AA. CYP2 enzymes express mainly an epoxygenase activity, and CYP2B, 2C, and 2J are the major epoxygenases that metabolize AA into EETs (8). CYP1 enzymes that metabolize xenobiotics can also metabolize endobiotics such as steroid hormones, retinoids and fatty acids (21-24). CYP1A1 and CYP1B1 are expressed in several extrahepatic tissues, including cardiovascular tissues (25). CYP1A1-encoded enzymes are expressed in vascular endothelium and smooth muscle cells, with much higher levels of activity in endothelial cells, whereas CYP1B1 is highly expressed in VSMCs, with little expression in endothelial cells (26). However, shear stress upregulates mRNA and protein levels of CYP1A1 and CYP1B1 in endothelial cells (27). CYP1B1 can also metabolize AA *in vitro* mainly into midchain HETEs and to a lesser degree into terminal HETEs and EETs (24). Moreover, bioactivation of procarcinogenic compounds such as aromatic hydrocarbons by CYP1B1 leads to formation of intermediates that form DNA adducts and polycyclic biphenyls which uncouple CYP1B1 resulting in generation of ROS, lipid peroxidation and DNA oxidation (28,29). Recently, however, we have shown that CYP1B1 mediates Ang II-induced VSMC migration, and protein synthesis through ROS production (30). Therefore, it is possible that CYP1B1, through generation of AA metabolites and/or ROS, might activate one or more of the signaling molecules (e.g., ERK1/2, p38 MAPK) that contribute to Ang II-induced hypertension. To test this hypothesis, we examined the effects of 2,3′,4,5′-tetramethoxystilbene (TMS), a selective inhibitor of CYP1B1 (31), on the development and maintenance of Ang II-induced hypertension in rats and the effects of Ang II in wild-type (*Cyp1b1*$^{+/+}$) and CYP1B1 knockout (*Cyp1b1*$^{-/-}$) mice. The results of this study demonstrate that CYP1B1 contributes to the development and maintenance of hypertension, most likely by increased generation of ROS, ERK1/2, and p38 MAPK activity, vascular hypertrophy, endothelial dysfunction, and increased vascular reactivity.

Methods

Please see the online Data Supplement at http://hyper.ahajournals.org

Results

TMS counteracts the hypertensive effect of Ang II in rats

Ang II infusion increased MAP that was prevented by TMS; TMS alone did not alter MAP (Figure S1A). In hypertensive rats injected with TMS, MAP returned to basal levels (Figure S1B). For each treatment, appropriate vehicles were administered and no difference was observed (data not shown).

TMS protects against cardiac hypertrophy, fibrosis and inflammation associated with Ang II-induced hypertension in rats

Ang II infusion increased heart-to-body weight (HW/BW) ratio and BNP mRNA expression, indicators of cardiac hypertrophy. TMS alone had no effect on HW/BW ratio and BNP mRNA level but minimized the increase in these parameters caused by Ang II (Table S1). The increase in myofibroblasts, as indicated by α-smooth muscle actin staining in the myocardium, and ED-1 positive cells, an index of macrophages, in the perivascular space of the heart caused by Ang II was reduced by TMS (Figure S2).

TMS decreases CYP1B1 activity but not its expression or plasma levels of 12- and 20-HETE

CYP1B1 protein expression was not altered in the aorta (Figure S3A) or heart (Figure S3B) following the various treatments. However, in animals treated with TMS, CYP1B1 activity in the aorta and heart was reduced. CYP1B1 activity was not increased in animals infused with Ang II but was reduced by concurrent treatment with TMS (Figures S3C, S3D). In hypertensive rats given TMS, CYP1B1 activity was decreased in the aorta and heart (Figure S4). Plasma levels of 12- and 20-HETE were not changed in the different treatment groups (Table S2).

TMS prevents increased vascular reactivity and vascular smooth muscle hypertrophy and improves endothelial dysfunction in Ang II-treated rats

Ang II-induced hypertension was associated with an increased response of aorta (Figure S5A) and mesenteric (Figure S5B) and femoral (Figure S5C) arteries to phenylephrine (PE), endothelin-1 (ET-1), vasopressin (VP), as well as media: lumen ratio of these vessels (Table S3); these increases were prevented in rats treated with TMS. In rats given TMS alone, responses of these vessels to the above agents and their media: lumen ratio was not altered (Figures S5A-S5C and Table S3, respectively). In hypertensive rats given TMS, both vascular reactivity and media: lumen ratio were reduced in all blood vessels studied (Figure S6 and Table S3, respectively).

Ang II infusion caused endothelial dysfunction in the aorta and femoral artery, but not the mesenteric artery, as determined by the dilatory effect of ACh (Figure S7A). In animals infused with Ang II and given TMS, ACh- and SNP-induced relaxations were not altered (Figures S7A, S7B). Following treatment with TMS to animals after they were made hypertensive with Ang II, ACh- and SNP-induced relaxations were not altered (Figure S8).

TMS inhibits ROS production, activity and expression of NADPH oxidase (NOX 1) and ERK1/2 and p38 MAPK activities in the rat aorta caused by Ang II infusion

ROS production, as determined by dihydroethidium (DHE) fluorescence, was increased in the aorta of animals infused with Ang II (Figures S9A, S9B). TMS treatment alone decreased ROS production in the aorta and abolished the increase caused by Ang II infusion (Figures S9A, S9B). To confirm the DHE fluorescence caused by generation of 2-hydroxyethidium (2-OHE), as a specific indicator of superoxide production, we employed a HPLC method to separate 2-OHE formed from DHE generated in isolated aortic rings and measured using a fluorescence detector. As was observed with the DHE fluorescence, animals infused with Ang II showed a

greater conversion of DHE to 2-OHE, which was inhibited with TMS (Figure S9C). TMS treatment alone had a minimal effect on basal conversion of DHE to 2-OHE (Figure S9C). ROS production was also decreased in animals made hypertensive and then given TMS, as determined by fluorescence microscopy after exposure of vessels to DHE (Figure S10). Expression of NOX 1, as measured by western blot analysis (Figure S11A), and NADPH oxidase activity, as measured by a lucigenin-based luminescence assay, were increased in the aorta of Ang II-infused animals but inhibited in animals treated with TMS (Figure S11B). Expression of NOX 4 was not altered in any treatment group (Figure S11C).

Ang II is known to increase the activity of ERK1/2 and p38 MAPK in VSMCs, which contribute to hypertrophy and increased vascular reactivity. In this study, ERK1/2 and p38 MAPK activity, measured by phosphorylation of these kinases, were increased in the aorta of rats infused with Ang II that was attenuated in rats treated with TMS (Figure S12).

CYP1B1 contributes to the development of Ang II-induced hypertension in mice

To further determine the contribution of CYP1B1 in the development of Ang II-induced hypertension, we examined the effect of Ang II in *Cyp1b1*$^{-/-}$ and *Cyp1b1*$^{+/+}$ mice. Infusion of Ang II increased MAP in *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice, but the increase was significantly less in *Cyp1b1*$^{-/-}$ than in *Cyp1b1*$^{+/+}$ mice (Figure 1A). In *Cyp1b1*$^{+/+}$ mice, TMS treatment alone had no effect on MAP, but the Ang II-induced increase in MAP was prevented by TMS (Figure 1B). The CYP1B1-independent component of Ang II-induced hypertension in *Cyp1b1*$^{-/-}$ mice was also abolished by TMS (Figure 1C).

TMS and CYP1B1 gene disruption reduce cardiac hypertrophy and CYP1B1 activity in Ang II-induced hypertension in mice

Infusion of Ang II increased HW/BW ratio in *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice; however, the increase in HW/BW ratio was significantly less in *Cyp1b1*$^{-/-}$ mice. In both strains of mice, the increase in HW/BW ratio associated with Ang II was prevented by TMS (Table 1).

CYP1B1 activity in the heart and kidney of *Cyp1b1*$^{-/-}$ mice was inhibited (Figures S13A, S13B). Infusion of Ang II did not increase CYP1B1 activity in tissues from *Cyp1b1*$^{-/-}$ or *Cyp1b1*$^{+/+}$ mice; however, CYP1B1 activity was further reduced by TMS in both strains of mice (Figures S13A, S13B). The remaining activity in *Cyp1b1*$^{-/-}$ mice could result from CYP1A1 activity detected by the assay. *Cyp1b1*$^{-/-}$ mice showed no expression of CYP1B1 in the heart and infusion of Ang II did not alter CYP1B1 protein expression in the heart of *Cyp1b1*$^{+/+}$ mice (Figure S13C).

Ang II-induced increase in vascular reactivity and hypertrophy and endothelial dysfunction are diminished in *Cyp1b1*$^{-/-}$ mice

In *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice, infusion of Ang II increased vascular reactivity of the aorta to PE and ET-1 and media: lumen ratio; these increases were significantly less in *Cyp1b1*$^{-/-}$ than in *Cyp1b1*$^{+/+}$ mice (Figures 2A, B and Table 2, respectively). The increased vascular reactivity to PE and ET-1 and media: lumen ratio associated with Ang II in *Cyp1b1*$^{+/+}$ mice and that remaining in *Cyp1b1*$^{-/-}$ mice was prevented by TMS; TMS alone had no effect on these parameters (Figures S14A-S14D and Table 2).

Ang II infusion resulted in endothelial dysfunction, as indicated by decreased relaxation to ACh in the aorta from *Cyp1b1*$^{+/+}$ but not *Cyp1b1*$^{-/-}$ mice (Figure S15A). Endothelium-independent relaxation of aorta to SNP in *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice remained unaltered in all treatment groups (Figure S15B). The decrease in ACh-induced relaxation of aorta from *Cyp1b1*$^{+/+}$ mice infused with Ang II was prevented by TMS (Figure S15C); SNP-induced relaxation of the aorta was not altered (Figure S15D). In *Cyp1b1*$^{-/-}$ mice infused with Ang II,

ACh- and SNP-induced relaxation of the aorta remained unaltered (Figures S15E, S15F, respectively).

Ang II-induced increase in ROS production is diminished in *Cyp1b1*$^{-/-}$ mice and inhibited by TMS in *Cyp1b1*$^{+/+}$ mice

Infusion of Ang II in both *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice increased aortic ROS production, as measured by fluorescence of 2-OHE generated after exposure of aorta to DHE, but the increase in fluorescence in *Cyp1b1*$^{-/-}$ mice was significantly less than that in *Cyp1b1*$^{+/+}$ mice (Figure 3). In both *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice infused with Ang II or its vehicle, TMS treatment decreased the intensity of fluorescence (Figure 3).

Ang II-induced increase in expression of NOX 1 and NADPH oxidase activity is diminished in the heart of *Cyp1b1*$^{-/-}$ mice

Ang II infusion increased expression of NOX 1 in the heart of of *Cyp1b1*$^{+/+}$ mice; this increase was significantly greater than that observed in *Cyp1b1*$^{-/-}$ mice (Figure S16A). In addition, NADPH oxidase activity was increased in the hearts of C*yp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice, but the increase in *Cyp1b1*$^{-/-}$ mice was significantly less than that in C*yp1b1*$^{+/+}$ mice (Figure S16B).

Discussion

This is the first study to demonstrate a novel mechanism whereby CYP1B1 contributes to development and maintenance of Ang II-induced hypertension and associated vascular hypertrophy, endothelial dysfunction, increased vascular reactivity to vasoconstrictor agents, cardiac hypertrophy, fibrosis and inflammation and generation of ROS, increased expression of NOX 1 and activities of NADPH oxidase, ERK1/2 and p38MAPK.

Our finding that TMS, a selective inhibitor of CYP1B1 (31), decreased CYP1B1 activity in the rat aorta and heart, prevented Ang II-induced increase in MAP and normalized the MAP raised by Ang II infusion, and minimized the associated increase in a) HW/BW ratio and the expression of BNP mRNA in the left ventricle; b) actin staining of myofibroblasts and accumulation of ED-1-positive cells in the myocardium; c) the response of the aorta and mesenteric and femoral arteries to PE, ET-1, VP, and the media: lumen ratio of these vessels, suggests that CYP1B1 contributes to the development and maintenance of hypertension and cardiac hypertrophy, fibrosis and inflammation, and vascular reactivity and hypertrophy. Because TMS treatment also reduced CYP1B1 activity in the heart and aorta of vehicle-treated animals and Ang II infusion did not increase the activity or expression of CYP1B1, it appears to be constitutively active in these tissues as shown in VSMCs (30). Although TMS treatment prevented the development and maintenance of hypertension caused by Ang II in rats, it did not completely inhibit CYP1B1 activity in the aorta or heart; the remaining activity could result from CYP1A1 activity detected by our assay or a related enzyme. In cultured rat VSMCs that do not express CYP1A1, TMS blocks the activity of CYP1B1 as measured by our assay (30).

Recombinant CYP1B1 can metabolize AA *in vitro* into midchain and terminal HETEs, including 12- and 20-HETE (24). Both 12- and 20-HETE contribute to VSMC proliferation and/or hypertrophy and vascular contraction caused by Ang II, and 20-HETE increases reactivity of mesenteric arteries to PE from SHR and WKY rats (32). Therefore, it is possible that the increased vascular reactivity and hypertrophy caused by Ang II could result from increased production of eicosanoids, 12- and 20-HETE. However, this appears to be unlikely, because plasma levels of 12- and 20-HETE were not altered in rats treated with Ang II or TMS. In rat VSMC, TMS or adenovirus CYP1B1 shRNA also do not alter AA metabolism into HETEs (30). The conversion of AA to 12- and 20-HETE, examined in the femoral arteries of rats treated with TMS was also not altered (our unpublished data).

In the present study, infusion of Ang II in rats caused endothelial dysfunction in the aorta and femoral artery, as indicated by attenuation of relaxation to ACh, but not to SNP that acts directly on vascular smooth muscle. Inasmuch as loss of ACh-induced relaxation caused by Ang II infusion was restored by concurrent administration of TMS to rats, it appears that CYP1B1 participates in endothelial dysfunction in the aorta and femoral artery and may also contribute to increased vascular reactivity in Ang II-induced hypertension. Endothelium-dependent relaxation depends mainly on NO in larger arteries and on endothelium-derived hyperpolarizing factor(s) (EDHF) in small vessels (33). Chronic infusion of Ang II reduces NO-mediated relaxation in the aorta (34), whereas Ang II-infusion for 21 but not 14 days produces loss of the major component of relaxation dependent on EDHF and not the minor NO-dependent component of relaxation in rat superior mesenteric artery (35). In our study, infusion of Ang II also failed to cause endothelial dysfunction in small resistance mesenteric artery. From these observations, it follows that endothelial dysfunction in different vascular tissues depends on the duration of exposure to Ang II and that NO-dependent relaxation in larger vessels is more sensitive to Ang II and depends on CYP1B1 activity.

The endothelial dysfunction in different models of hypertension is in part due to the result of inactivation of NO by ROS (34). Therefore, in our study, restoration of endothelium-dependent relaxation to ACh in the aorta and femoral artery, produced by concurrent treatment with TMS in Ang II-infused rats, most likely results from decreased production of ROS generated via CYP1B1. Supporting this view was our demonstration that Ang II infusion increased aortic superoxide production and expression of NOX-1 and NADPH oxidase activity, which was prevented in the aorta of rats treated with TMS. Recently, we reported that Ang II-induced increase in ROS production in rat VSMCs is mediated by CYP1B1 (30). Because Ang II is known to stimulate ROS production by activating NADPH oxidase and it has been implicated in Ang II-induced hypertension (17,36), it is possible that ROS and/or AA metabolites generated by CYP1B1, independent of HETEs, result in activation of NADPH oxidase. ROS have been reported to amplify their own production by activating NADPH oxidases, xanthine oxidase, increasing intracellular uptake of iron and/or uncoupling endothelial nitric oxide synthase (37). Further studies are required to determine the relationship between CYP1B1 and NOX and other ROS-producing systems. Moreover, we cannot exclude the possibility of an additional direct effect of TMS on NADPH or other ROS-producing systems.

The increase in ROS production from AA via CYP1B1 by Ang II infusion could result in increased vascular reactivity and hypertrophy through activation of ERK1/2 and p38 MAPK that are known to mediate Ang II-induced hypertrophy of cultured VSMCs (10,38). Supporting this view was our finding that infusion of Ang II increased aortic ERK1/2 and p38 MAPK activity that was attenuated by treatment with TMS. Moreover, we have shown that, in cultured rat VSMC or in cells transduced with adenovirus CYP1B1 shRNA, Ang II- and AA-induced ERK1/2 and p38 MAPK are inhibited by TMS without alterations in expression of AT1 receptor or its coupling to G proteins (30).

Further evidence that CYP1B1 contributes to the development of Ang II-induced hypertension and associated pathophysiological changes is derived from our studies in *Cyp1b1*$^{-/-}$ mice that do not express CYP1B1 protein. Our findings that the increase in MAP, HW/BW ratio, media: lumen ratio, the response to PE and ET-1, ROS production and cardiac expression of NOX 1 and NADPH oxidase activity caused by Ang II infusion in *Cyp1b1*$^{+/+}$ mice were significantly decreased in *Cyp1b1*$^{-/-}$ mice strongly support our findings in the rat treated with TMS that CYP1B1 contributes to Ang II-induced hypertension and associated pathophysiologial changes. Our finding that endothelial function remained unaltered in *Cyp1b1*$^{-/-}$ mice indicates that CYP1B1 is required for aortic endothelial dysfunction associated with Ang II-induced hypertension. CYP1B1 activity, measured in the heart and kidney of *Cyp1b1*$^{-/-}$ mice, was reduced but not abolished and was further reduced by TMS. The remaining activity could result

from CYP1A1 or other related enzyme(s), as mentioned above. Inasmuch as administration of TMS abolished Ang II-induced increase in blood pressure and all the associated cardiovascular changes in *Cyp1b1*$^{+/+}$ mice, as well as the CYP1B1-independent component of Ang II actions in *Cyp1b1*$^{-/-}$ mice, it appears that TMS exerts an additional protective effect against the deleterious effects of Ang II on the cardiovascular system. The mechanism of the protective effects of TMS against the CYP1B1-independent component of Ang II-induced hypertension and associated ROS generation and vascular changes which could be due to its direct effect on one or more ROS generating systems remains to be determined.

Ang II-induced hypertension also depends upon its actions in the kidney (39), and CNS, and superoxides have been implicated in hypertension caused by central actions of Ang II (40). Recently, it has been shown that T cells (Th 17 cells), via generation of superoxides, participate in Ang II-induced hypertension (41). Another recent report shows that Ang II, by increasing RhoA activity via Jak2-induced phosphorylation of Rho exchange factor Arhgef1 (42), results in increased vascular contraction and hypertension. Therefore, it is possible that CYP1B1 expressed in the kidney, brain (43) and lymphocytes (44), via ROS generation and/or increased RhoA activity, might also contribute to Ang II-induced hypertension. Alternatively, one or more of these mechanisms might contribute to the CYP1B1-independent and TMS-sensitive component of Ang II-induced hypertension and associated pathophysiological changes. Whether CYP1B1 directly or indirectly also contributes to hypertension caused by increased activity of the sympathetic nervous system also remains to be determined.

Perspectives

Increased activity of the renin-angiotensin system is a major contributing factor in the development of various vascular diseases including hypertension. Further understanding of the mechanisms that are involved in Ang II-dependent hypertension would provide a rationale approach for the development new therapeutic agents for the treatment of cardiovascular diseases. This study provides evidence for the first time that CYP1B1 contributes to Ang II-induced hypertension and associated pathophysiological changes including increased ROS production, vascular reactivity, endothelial dysfunction, and vascular and cardiac hypertrophy. In preliminary experiments, we have found that CYP1B1 also contributes to DOCA/Salt-, N^{ω}-nitro-$_{L}$-arginine methyl ester hydrochloride-induced hypertension and in spontaneous hypertension in rats. Moreover, TMS, which prevents both CYP1B1-dependent and -independent components of Ang II-induced hypertension and associated pathophysiological changes, could be clinically useful for treating hypertension and other cardiovascular and related inflammatory diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Bernd Meibohm and Josiah T. Ryman for assistance with the HPLC experiment and Dr. David L. Armbruster for editorial assistance.

Sources of Funding

The described project was supported by Grant R01-19134-34 (K.U.M.) from The National Institutes of Health, Heart, Lung and Blood Institutes. B.L. J. was supported in part by a fellowship from the Neuroscience Institute, University of Tennessee Health Science Center, Memphis. S.S.-F. was supported by the Scientific and Technical Research Council of Turkey (TUBITAK). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of HBLI.

References

1. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: From physiology to the pathophysiology of hypertension and kidney disease. Pharmacol Rev 2007;59:251–287. [PubMed: 17878513]
2. Muthalif MM, Benter IF, Uddin MR, Harper JL, Malik KU. Signal transduction mechanisms involved in angiotensin-(1–7)-stimulated arachidonic acid release and prostanoid synthesis in rabbit aortic smooth muscle cells. J Pharmacol Exp Ther 1998;284:388–398. [PubMed: 9435202]
3. Hao C-M, Breyer MD. Physiologic and pathophysiologic roles of lipid mediators in the kidney. Kidney Int 2007;71:1105–1115. [PubMed: 17361113]
4. McGiff JC. Prostaglandins, prostacyclin, and thromboxanes. Annu Rev Pharmacol Toxicol 1981;21:479–509. [PubMed: 7016014]
5. Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. Nat Rev Drug Discov 2009;8:794–805. [PubMed: 19794443]
6. Nasjletti A, Arthur C. Corcoran Memorial Lecture. The role of eicosanoids in angiotensin-dependent hypertension. Hypertension 1998;31:194–200. [PubMed: 9453302]
7. McGiff JC, Quilley J. 20-Hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids and blood pressure. Curr Opin Nephrol Hypertens 2001;10:231–237. [PubMed: 11224699]
8. Roman RJ. *P*-450 Metabolites of arachidonic acid in the control of cardiovascular function. Physiol Rev 2002;82:131–185. [PubMed: 11773611]
9. Nakao J, Ooyama T, Ito H, Chang W-C, Murota S. Comparative effect of lipoxygenase products of arachidonic acid on rat aortic smooth muscle cell migration. Atherosclerosis 1982;44:339–342. [PubMed: 6816252]
10. Reddy MA, Thimmalapura P-R, Lanting L, Nadler JL, Fatima S, Natarajan R. The oxidized lipid and lipoxygenase product 12(*S*)-hydroxyeicosatetraenoic acid induces hypertrophy and fibronectin transcription in vascular smooth muscle cells via p38 MAPK and cAMP response element-binding protein activation. Mediation of angiotensin II effects. J Biol Chem 2002;277:9920–9928. [PubMed: 11786549]
11. Natarajan R, Gonzales N, Lanting L, Nadler J. Role of the lipoxygenase pathway in angiotensin II-induced vascular smooth muscle cell hypertrophy. Hypertension 1994;23:I142–I147. [PubMed: 8282345]
12. Uddin MR, Muthalif MM, Karzoun NA, Benter IF, Malik KU. Cytochrome P-450 metabolites mediate norepinephrine-induced mitogenic signaling. Hypertension 1998;31:242–247. [PubMed: 9453310]
13. Stec DE, Gannon KP, Beaird JS, Drummond HA. 20-Hydroxyeicosatetraenoic acid (20-HETE) stimulates migration of vascular smooth muscle cells. Cell Physiol Biochem 2007;19:121–128. [PubMed: 17310106]
14. Alonso-Galicia M, Maier KG, Greene AS, Cowley AW Jr. Roman RJ. Role of 20-hydroxyeicosatetraenoic acid in the renal and vasoconstrictor actions of angiotensin II. Am J Physiol Regul Integr Comp Physiol 2002;283:R60–R68. [PubMed: 12069931]
15. Nozawa K, Tuck ML, Golub M, Eggena P, Nadler JL, Stern N. Inhibition of lipoxygenase pathway reduces blood pressure in renovascular hypertensive rats. Am J Physiol Heart Circ Physiol 1990;259:H1774–H1780.
16. Muthalif MM, Karzoun NA, Gaber L, Khandekar Z, Benter IF, Saeed AE, Parmentier J-H, Estes A, Malik KU. Angiotensin II–induced hypertension. Contribution of Ras GTPase/mitogen-activated protein kinase and cytochrome P450 metabolites. Hypertension 2000;36:604–609. [PubMed: 11040243]
17. Griendling K, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141–1148. [PubMed: 8187280]
18. Viedt C, Soto U, Krieger-Brauer HI, Fei J, Elsing C, Kübler W, Kreuzer J. Differential activation of mitogen-activated protein kinases in smooth muscle cells by angiotensin II. Involvement of p22phox and reactive oxygen species. Arterioscler Thromb Vasc Biol 2000;20:940–948. [PubMed: 10764657]

19. Touyz RM, Yao G, Viel E, Amiri F, Schiffrin EL. Angiotensin II and endothelin-1 regulate MAP kinases through different redox-dependent mechanisms in human vascular smooth muscle cells. J Hypertens 2004;22:1141–1149. [PubMed: 15167449]
20. Bao W, Behm DJ, Nerurkar SS, Ao Z, Bentley R, Mirabile RC, Johns DG, Woods TN, Doe CPA, Coatney RW, Ohlstein JF, Douglas SA, Willette RN, Yue T-L. Effects of p38 MAPK Inhibitor on angiotensin II-dependent hypertension, organ damage, and superoxide anion production. J Cardiovasc Pharmacol 2007;49:362–368. [PubMed: 17577100]
21. Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 1996;56:2979–2984. [PubMed: 8674051]
22. Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci 2004;95:1–6. [PubMed: 14720319]
23. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc Natl Acad Sci U S A 1996;93:9776–9781. [PubMed: 8790407]
24. Choudhary D, Jansson I, Stoilov I, Sarfarazi M, Schenkman JB. Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1B1. Drug Metab Dispos 2004;32:840–847. [PubMed: 15258110]
25. Korashy HM, El-Kadi AOS. The role of aryl hydrocarbon receptor in the pathogenesis of cardiovascular diseases. Drug Metab Rev 2006;38:411–450. [PubMed: 16877260]
26. Zhao W, Parrish AR, Ramos KS. Constitutive and inducible expression of cytochrome P450IA1 and P450IB1 in human vascular endothelial and smooth muscle cells. In Vitro Cell Dev Biol Anim 1998;34:671–673. [PubMed: 9794216]
27. Conway DE, Sakurai Y, Weiss D, Vega JD, Taylor WR, Jo H, Eskin SG, Marcus CB, McIntire LV. Expression of CYP1A1 and CYP1B1 in human endothelial cells: regulation by fluid shear stress. Cardiovasc Res 2009;81:669–677. [PubMed: 19126602]
28. Moorthy B, Miller KP, Jiang W, Ramos KS. The atherogen 3-methylcholanthrene induces multiple DNA adducts in mouse aortic smooth muscle cells: role of cytochrome P4501B1. Cardiovasc Res 2002;53:1002–1009. [PubMed: 11922910]
29. Green RM, Hodges NK, Chipman JK, O'Donovan MR, Graham M. Reactive oxygen species from the uncoupling of human cytochrome P450 1B1 may contribute to the carcinogenicity of dioxin-like polychlorinated biphenyls. Mutagenesis 2008;23:457–463. [PubMed: 18583386]
30. Yaghini FA, Song CY, Lavrentyev EN, Ghafoor HUB, Fang XR, Estes AM, Campbell WB, Malik KU. Angiotensin II-induced vascular smooth muscle cell migration and growth are mediated by cytochrome P450 1B1-dependent superoxide generation. Hypertension 2010;55:1461–1467. [PubMed: 20439821]
31. Chun Y-J, Kim S, Kim D, Lee S-K, Guengerich FP. A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. Cancer Res 2001;61:8164–8170. [PubMed: 11719446]
32. Zhang F, Wang M-H, Krishna UM, Falck JR, Laniado-Schwartzman M, Nasjletti A. Modulation by 20-HETE of phenylephrine-induced mesenteric artery contraction in spontaneously hypertensive and Wistar-Kyoto rats. Hypertension 2001;38:1311–1315. [PubMed: 11751709]
33. Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M, Takeshita A. The importance of the hyperpolarizing mechanism increases as the vessel size decreases in endothelium-dependent relaxations in rat mesenteric circulation. J. Cardiovasc Pharmacol 1996;28:703–711. [PubMed: 8945685]
34. Sarr M, Chataigneau M, Martins S, Schott C, El Bedoui J, Oak M-H, Muller B, Chataigneau T, Schini-Kerth VB. Red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats: role of NADPH oxidase. Cardiovasc Res 2006;71:794–802. [PubMed: 16822492]
35. Dal-Ros S, Bronner C, Schott C, Kane MO, Chataigneau M, Schini-Kerth VB, Chataigneau T. Angiotensin II-induced hypertension is associated with a selective inhibition of endothelium-derived hyperpolarizing factor-mediated responses in the rat mesenteric artery. J Pharmacol Exp Ther 2009;328:478–486. [PubMed: 18984652]

36. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. 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;97:1916–1923. [PubMed: 8621776]

37. Cai H. NAD(P)H oxidase–dependent self-propagation of hydrogen peroxide and vascular disease. Circ Res 2005;96:818–822. [PubMed: 15860762]

38. El Mabrouk M, Touyz RM, Schiffrin EL. Differential ANG II-induced growth activation pathways in mesenteric artery smooth muscle cells from SHR. Am J Physiol Heart Circ Physiol 2001;281:H30–H39. [PubMed: 11406465]

39. Crowley SD, Gurley SB, Oliverio MI, Pazmino AK, Griffiths R, Flannery PJ, Spurney RF, Kim H-S, Smithies O, Le TH, Coffman TM. Distinct roles for the kidney and systemic tissues in blood pressure regulation by the renin-angiotensin system. J Clin Invest 2005;115:1092–1099. [PubMed: 15841186]

40. Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, Spitz DR, Davisson RL. Superoxide mediates the actions of angiotensin II in the central nervous system. Circ Res 2002;91:1038–1045. [PubMed: 12456490]

41. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II–induced hypertension and vascular dysfunction. J Exp Med 2007;204:2449–2460. [PubMed: 17875676]

42. Guilluy C, Brégeon J, Toumaniantz G, Rolli-Derkinderen M, Retailleau K, Loufrani L, Henrion D, Scalbert E, Bril A, Torres RM, Offermanns S, Pacaud P, loirand G. The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. Nat Med 2010;16:183–190. [PubMed: 20098430]

43. Rieder CRM, Ramsden DB, Williams AC. Cytochrome P450 1B1 mRNA in the human central nervous system. J Clin Pathol: Mol Pathol 1998;51:138–142.

44. Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW, Walker NJ. Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-*p*dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. Cancer Epidemiol Biomarkers Prev 1999;8:139–146. [PubMed: 10067811]

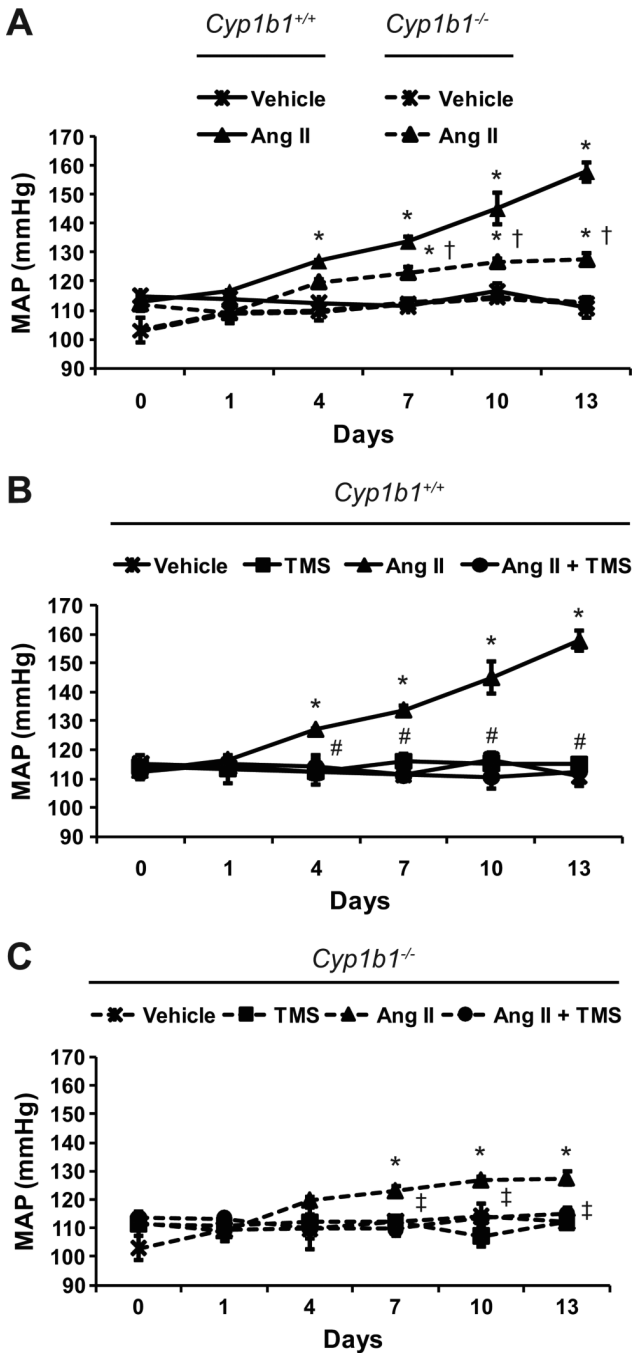

Figure 1. CYP1B1 contributes to the development of Ang II-induced hypertension in mice

(A) *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice were infused with either Ang II (1000 μg/kg/min) or vehicle with miniosmotic pumps for 13 days, and blood pressure was measured by tail cuff every 3rd day. **(B)** *Cyp1b1*$^{+/+}$ mice and **(C)** *Cyp1b1*$^{-/-}$ mice were infused with either Ang II (1000 μg/kg/min) or vehicle and given i.p. injections of the CYP1B1 inhibitor, TMS (300 μg/kg), every 3rd day starting day 1 of the experiment, and blood pressure was measured every 3rd day. *P < 0.05 vehicle vs. corresponding value from Ang II-treated animal; $^{\dagger}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II; $^{\#}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{+/+}$ Ang II + TMS; $^{\ddagger}P < 0.05$ *Cyp1b1*$^{-/-}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II + TMS. (n = 4 for all experiments, and data are expressed as mean ± SEM).

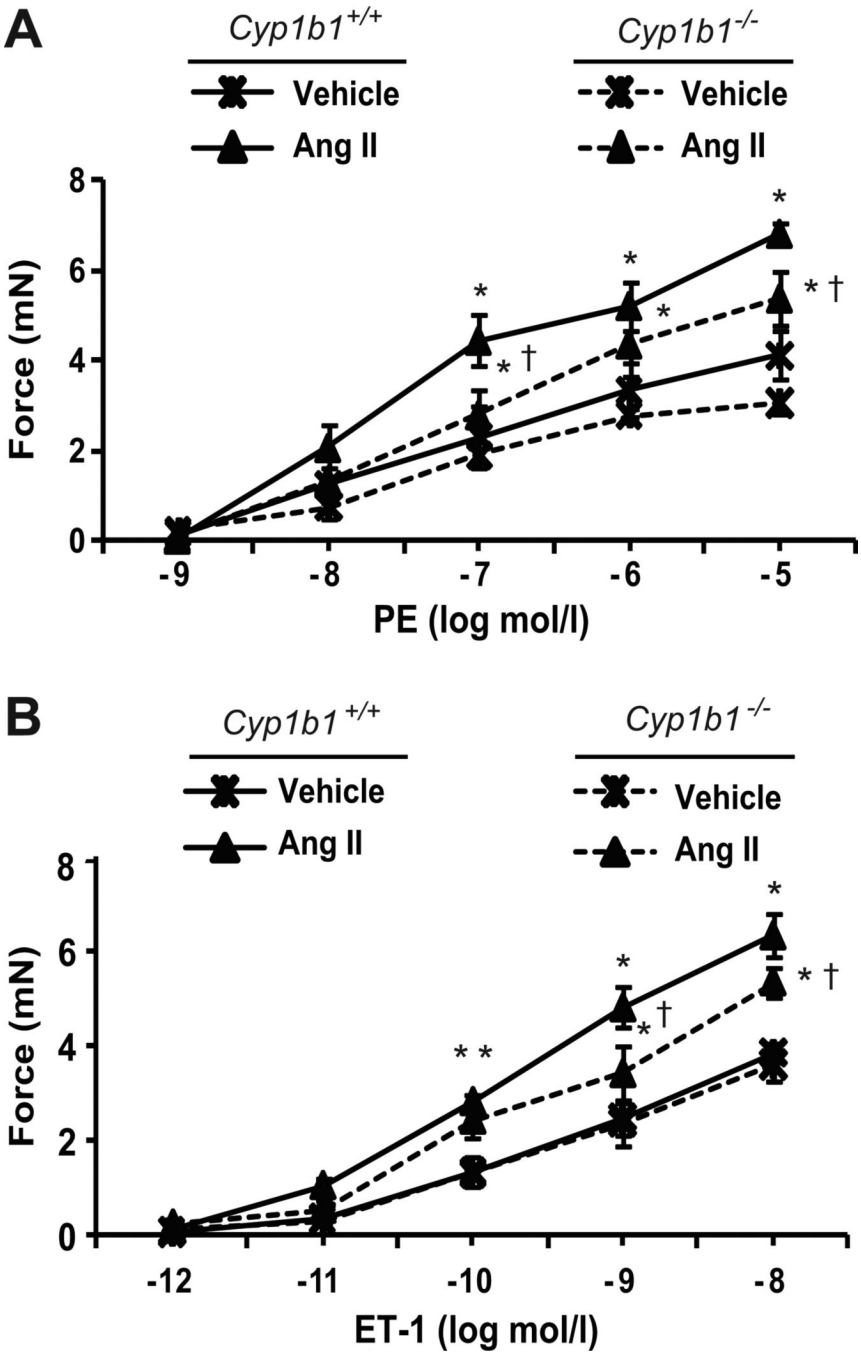

Figure 2. TMS and *Cyp1b1* gene disruption reduce increased aortic response to vasoconstrictor agents associated with Ang II-induced hypertension in mice

Cyp1b1$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice were infused with either Ang II or vehicle for 13 days and given i.p. injections of TMS as described in Figure 1 legend. Vascular reactivity was measured in the aorta as described in Methods. **(A, B)** The response of aorta of *Cyp1b1*$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice infused with Ang II or vehicle to increasing concentrations of phenylephrine (PE) and endothelin-1 (ET-1). **P* < 0.05 vehicle vs. corresponding value from Ang II-treated animal; †*P* < 0.05 *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II. (n = 4 for all experiments, and data are expressed as mean ± SEM).

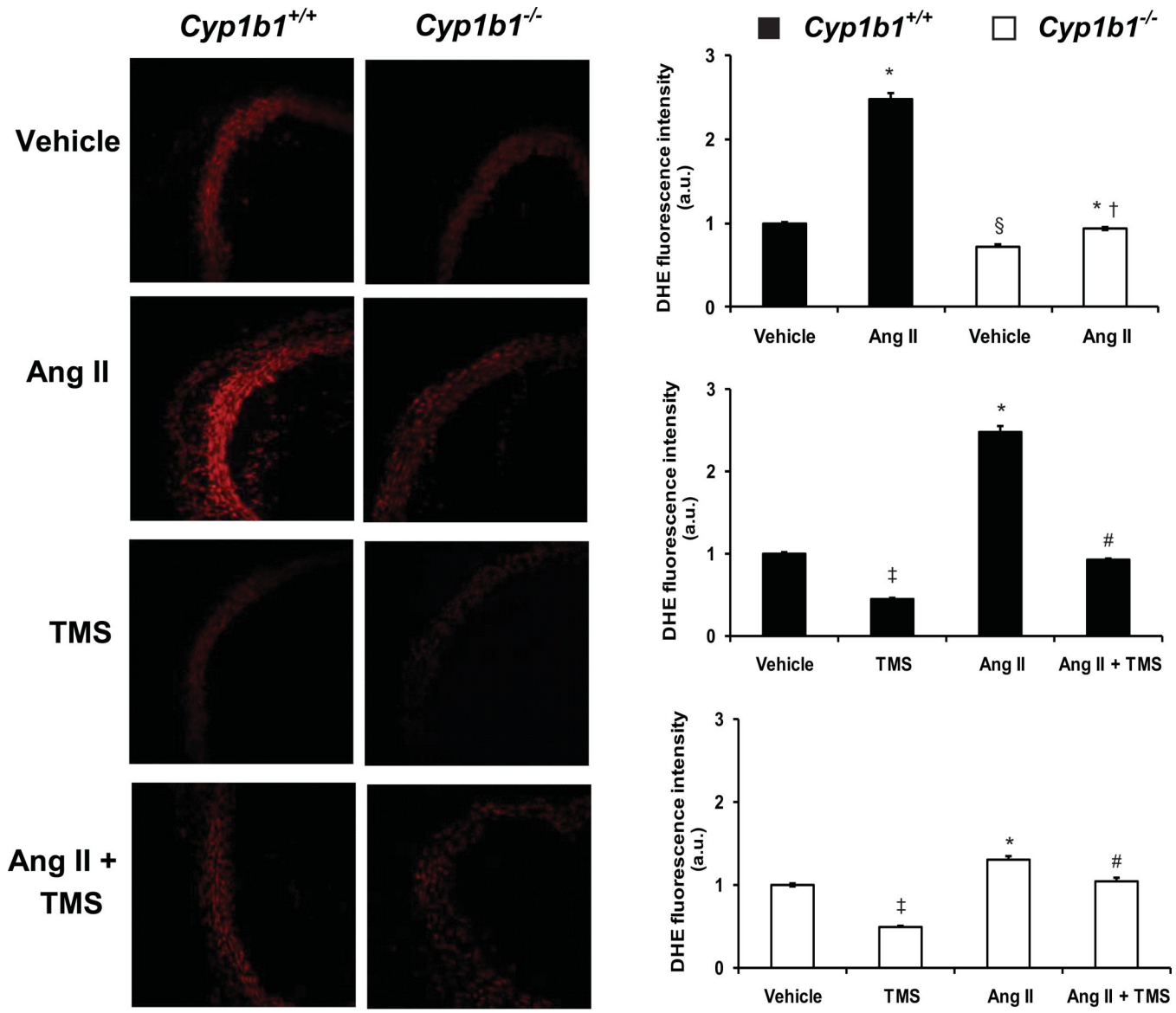

Figure 3. TMS and *Cyp1b1* gene disruption reduce vascular oxidative stress associated with Ang II-induced hypertension in mice

Cyp1b1$^{+/+}$ and *Cyp1b1*$^{-/-}$ mice were infused with either Ang II or vehicle for 13 days and given i.p. injections of TMS as described in Figure 1 legend. ROS production was determined in aortic sections by DHE fluorescence as described in Methods. $^{\S}P < 0.05$ *Cyp1b1*$^{+/+}$ vehicle vs. *Cyp1b1*$^{-/-}$ vehicle; $^{*}P < 0.05$ vehicle vs. corresponding value from Ang II-treated animal; $^{\dagger}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II; $^{\ddagger}P < 0.05$ vehicle vs. corresponding value from TMS-treated animal; $^{\#}P < 0.05$ Ang II vs. corresponding value from Ang II + TMS-treated animal. (n = 4 for all experiments, and data are expressed as mean ± SEM).

Table 1

TMS and *Cyp1b1* gene disruption reduce cardiac hypertrophy associated with Ang II-induced hypertension in mice

Mouse		Vehicle	Ang II	TMS	Ang II + TMS
Cyp1b1$^{+/+}$	Body weight (BW) (g)	24.2 ± 0.2	26.1 ± 0.2	25.2 ± 0.8	26.4 ± 0.3
	Heart weight (HW) (mg)	112.7 ± 3.1	165.0 ± 3.5*	123.7 ± 3.5	127.7 ± 3.8‡
	HW (mg)/BW (g)	4.7 ± 0.2	6.3 ± 0.1*	4.9 ± 0.3	4.8 ± 0.1‡
Cyp1b1$^{-/-}$	Body weight (BW) (g)	25.6 ± 0.8	26.8 ± 0.1	25.5 ± 0.5	27.4 ± 0.6
	Heart weight (HW) (mg)	112.0 ± 4.1	139.7 ± 2.3*†	115.7 ± 3.9	123.0 ± 3.6§
	HW (mg)/BW (g)	4.4 ± 0.2	5.2 ± 0.1*†	4.5 ± 0.1	4.5 ± 0.1§

Animals were treated and the ratio of heart weight to body weight was calculated as described in Methods.

$^{*}P < 0.05$ vehicle vs. corresponding value from Ang II-treated animal

$^{†}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II

$^{‡}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{+/+}$ Ang II + TMS

$^{§}P \pm 0.05$ *Cyp1b1*$^{-/-}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II + TMS. (n = 4 for all experiments, and data are expressed as mean ± SEM).

Table 2

TMS and *Cyp1b1* gene disruption reduce media: lumen ratio associated with Ang II-induced hypertension in mice.

Thoracic aorta	Vehicle	Ang II	TMS	Ang II + TMS
Cyp1b1$^{+/+}$	6.14 ± 0.09	9.83 ± 0.59*	6.34 ± 0.45	6.27 ± 0.08‡
Cyp1b1$^{-/-}$	5.53 ± 0.93	7.09 ± 0.23*†	5.85 ± 0.58	5.96 ± 0.39§

Animals were treated and the media: lumen ratio of the aorta was calculated as described in Methods.

$^{*}P < 0.05$ vehicle vs. corresponding value from Ang II-treated animal

$^{†}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II

$^{‡}P < 0.05$ *Cyp1b1*$^{+/+}$ Ang II vs. *Cyp1b1*$^{+/+}$ Ang II + TMS

$^{§}P \pm 0.05$ *Cyp1b1*$^{-/-}$ Ang II vs. *Cyp1b1*$^{-/-}$ Ang II + TMS. (n = 4 for all experiments, and data are expressed as mean ± SEM).