Published in final edited form as:
Hypertension. 2010 November ; 56(5): 871–878. doi:10.1161/HYPERTENSIONAHA.110.154559.

CYP4A2-Induced Hypertension is 20-HETE and Angiotensin II-Dependent

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Abstract

We have previously shown that increased vascular endothelial expression of CYP4A2 leads to 20-HETE-dependent hypertension. The renin-angiotensin system (RAS) is a key regulator of blood pressure. In this study, we examined possible interactions between 20-HETE and RAS. In normotensive (110±3 mmHg) Sprague Dawley rats transduced with a lentivirus expressing the CYP4A2 cDNA under the control of an endothelial-specific promoter (VECAD-4A2), systolic blood pressure increased rapidly, reaching 139±1, 145±3 and 150±2 mmHg at 3, 5 and 10 days after transduction; blood pressure remained elevated, thereafter, with maximum levels of 163±3 mmHg. Treatment with lisinopril, losartan or the 20-HETE antagonist 20-hydroxyeicosa-6(Z), 15(Z)-dienoic acid (20-HEDE) decreased blood pressure to control values, but blood pressure returned to its high levels after cessation of treatment. Endothelial-specific overexpression of CYP4A2 resulted in increased expression of vascular angiotensin converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R) and increased levels of plasma and tissue Angiotensin II; all were attenuated by treatment with HET0016, an inhibitor of 20-HETE synthesis, or with 20-HEDE. In cultured endothelial cells, 20-HETE specifically and potently induced ACE expression without altering the expression of ACE2, angiotensinogen or angiotensin II receptors. This is the first study to demonstrate that 20-HETE, a key constrictor eicosanoid in the microcirculation, induces ACE and AT1R expression and increases Angiotensin II levels, suggesting that the mechanisms by which 20-HETE promotes hypertension include activation of RAS that is likely initiated at the level of ACE induction.

Keywords

Cytochrome P450; 20-HETE; ACE; Angiotensin II; Hypertension

INTRODUCTION

20-Hydroxyeicosatetraenoic acid (20-HETE) is a cytochrome P450 (CYP)-derived arachidonic acid metabolite formed by members of the CYP4 family. It is endowed with unique biological activities relevant to the regulation of vascular tone, renal function and

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Conflict(s) of Interest/Disclosure(s): None

blood pressure. The involvement of 20-HETE in hypertension has been significantly documented in animal models and humans. 20-HETE is a vasoconstrictor and natriuretic eicosanoid; hence, its contribution to the regulation of blood pressure depends on its sites of synthesis and action. In the vasculature, 20-HETE enhances the responsiveness to constrictor stimuli including pressure, oxygen, phenylephrine and endothelin-1, as well as inhibition of NO synthesis by mechanisms that include production of constrictor eicosanoids, inhibition of the smooth muscle cell large conductance Ca^{2+}-activated K^+ channel and the sensitization of the contractile apparatus to [Ca^{2+}] through phosphorylation of MLC20 [1]. We and others [2–4] demonstrated that, in circulatory districts other than the pulmonary, 20-HETE reduces endothelial-dependent vasorelaxation by uncoupling eNOS [5, 6]. In the kidney, 20-HETE biosynthesis is prominent in the proximal tubule and thick ascending limb. It promotes natriuresis by inhibiting Na^+/K^+-ATPase activity in the proximal tubule and inhibiting the Na^+-K^+-$2Cl^-$ co-transporter in the thick ascending limb [7]. Consequently, enhanced production of 20-HETE within the vasculature, as in the SHR [8, 9] or the androgen-dependent hypertensive rats [5, 10] and mice [11], or attenuated synthesis and action in renal tubules as in Dahl SS rats [12, 13] contribute to increases in blood pressure and hypertension. Accordingly, inhibition of vascular [5, 14] and induction of tubular 20-HETE synthesis [15] lowers blood pressure in experimental hypertensive models. In humans, decreased urinary excretion of 20-HETE has been shown to correlate with salt sensitivity of hypertension [16, 17], while increased urinary excretion of 20-HETE has been correlated with hypertension and with endothelial dysfunction [18, 19]. In all, these studies clearly implicated an important role for 20-HETE in the regulation of blood pressure.

The renin-angiotensin system (RAS) has been long recognized as a critical regulator of blood pressure and fluid homeostasis. Components of the RAS, including renin, angiotensin-converting enzyme (ACE), and angiotensin type 1 receptor (AT1R) are generally expressed throughout the body in tissues that impact blood pressure control. Angiotensin II (Ang II), the product of sequential degradation of angiotensinogen by renin and ACE and the final effector of the RAS system, increases blood pressure by mechanisms that include (i) vasoconstriction via AT1R activation in the vasculature and via increasing sympathetic tone and the release of arginine vasopressin, (ii) modulation of renal sodium and water reabsorption by stimulating renal AT1R, the production and release of aldosterone from the adrenal glands, or the sensation of thirst in the central nervous system. Blocking the synthesis or actions of Ang II lowers blood pressure in patients with hypertension. Equally, mice null for angiotensinogen, renin, ACE and $AT1_AR$ (the closest murine homologue to the human AT1R gene) exhibit a marked reduction in blood pressure, indicating the role of RAS in normal blood pressure homeostasis.

Given the characteristics of the RAS and the CYP4A-20-HETE pathways, interactions between these two systems are highly conceivable. To this end, Ang II has been shown to stimulate the release of 20-HETE in isolated preglomerular vessels [20]. Increased production of 20-HETE in the peripheral vasculature contributes to the acute vasoconstrictor response to Ang II [21, 22] and chronic inhibition of 20-HETE biosynthesis attenuates the development of Ang II-dependent hypertension [21, 23, 24]. All together, these studies suggest that 20-HETE may contribute to the hypertensive actions of Ang II. We have recently shown that gene transfer-mediated endothelial-specific CYP4A2 overexpression leads to 20-HETE-dependent hypertension in Sprague Dawley rats [25]. We used this model to examine a possible interplay between the CYP4A2-20-HETE pathway and RAS. Here, we show that 20-HETE-induced hypertension in the CYP4A2-transduced rats is associated with RAS upregulation and is abrogated by ACE inhibition or AT1R blockade. This is the first demonstration to suggest that 20-HETE's pro-hypertensive property is mediated, at least in part, by activation of the RAS, possibly through induction of vascular ACE.

METHODS AND MATERIALS

A detailed description of experimental protocols, methods and materials is included in a supplemental material (please see http://hyper.ahajournals.org).

RESULTS

20-HETE stimulates ACE expression in endothelial cells

A gene microarray was performed on mRNA extracted from cultured human endothelial cells treated with 20-HETE for 30 min. Among the few genes that were upregulated was ACE, which demonstrated a 5.76-fold increase compared to control (Table S1, Figure S1). No significant changes were observed in the expression of ACE-2, angiotensinogen, AT1R or AT2R (Figure S1). A real-time PCR analysis corroborated the microarray data; 20-HETE at a concentration of 5 nM increased ACE mRNA by 3-fold. 20-HETE-mediated induction of ACE mRNA was abolished by co-treatment with the 20-HETE antagonist 20-HEDE, demonstrating the specificity of the 20-HETE effect (Figure S1).

AT1 receptor antagonist reverses 20-HETE-mediated hypertension

The finding in cultured endothelial cells suggested the involvement of RAS in 20-HETE-mediated bioactions. Recently, we demonstrated that the lentivirus-mediated endothelial-specific transduction of CYP4A2 cDNA to Sprague Dawley rats resulted in hypertension that was offset by treatment with HET0016, indicating that this model displays 20-HETE-dependent hypertension [25]. In these rats, blood pressure significantly increased soon after injection of the lenti-VECAD-CYP4A2 and was greatly attenuated by daily treatment with HET0016 (Figure 1A). Blood pressure was not significantly altered in rats transduced with the lenti-VECAD-GFP (control transgene) treated with or without HET0016 (Figure 1B). At day 27 after infection, losartan was placed in the drinking water of untreated VECAD-CYP4A2-transduced rats. As seen in Figure 2A, losartan reduced blood pressure from 144±5 to 113±3 mmHg. Upon cessation (72 h after initiation) of losartan treatment, blood pressure returned to its high value (153±2 mmHg) and remained high till the end of the experiment (146±4 mmHg) (Figure 2A). Creatinine clearance decreased by 50% in the VECAD-CYP4A2-transduced rats when compared to the control VECAD-GFP-transduced rats. Treatment of VECAD-CYP4A2-transduced rats with HET0016 prevented the decrease in creatinine clearance (Figure 2C). The creatinine clearance data are indicative of reduced GFR consistent with the notion that 20-HETE is a renal vasoconstrictor and this action contributes to the hypertension in this model.

Endothelial CYP4A2 overexpression increases plasma and tissue levels of Ang II

The drop in blood pressure following losartan administration suggested a role for the RAS in mediating 20-HETE-dependent (HET0016-inhibitable) hypertension. Plasma renin concentration was not significantly different between control (VECAD-GFP-transduced), VECAD-CYP4A2-transduced and HET0016-treated VECAD-CYP4A2-transduced rats (Figure 2A). However, plasma Ang II levels were 3-fold higher in VECAD-CYP4A2-transduced rats when compared to control VECAD-GFP-transduced rats (Figure 2B). Moreover, treatment with HET0016 reduced plasma Ang II levels ($p=0.059$), suggesting that 20-HETE mediated this effect. Plasma Ang II levels in control rats (VECAD-GFP-transduced) treated with HET0016 or its vehicle amounted to 90±12 and 90±10 pg/ml, respectively (Figure 2B).

Similar results were obtained in aortic tissue. Ang II levels rose by 4-fold in aortas from VECAD-CYP4A2-transduced rats as compared to control (Figure 2C). Moreover, treatment of VECAD-CYP4A2-transduced rats with HET0016 reduced Ang II to levels not different

from control. Interestingly, analysis of renal tissues from the same groups of rats clearly showed no significant differences in the levels of tissue Ang II (Figure 2D).

Endothelial CYP4A2 overexpression increases vascular Ang II levels and expression of ACE and AT1R

Real-time PCR analysis of ACE and AT1R demonstrated significant increases in their mRNA levels in rats transduced with Lenti-VECAD-CYP4A2 when compared to rats transduced with the control lenti-VECAD-GFP; these increases were prevented by daily treatment with HET0016 (Figure 3A and B). In contrast, AT2R mRNA levels were not significantly altered by vascular CYP4A2 expression or 20-HETE levels (Figure 3C). Interestingly, treatment of control rats with HET0016 significantly reduced ACE mRNA levels as compared to untreated controls (Figure 4A).

ACE protein (somatic ACE ~170 KDa) levels increased by 2-fold in aorta of rats transduced with the VECAD-CYP4A2 as compared to control VECAD-GFP-transduced rats. Moreover, ACE protein levels were significantly decreased by treatment with HET0016 (Figure 3D), further indicating that 20-HETE contributed to the increased expression of ACE. AT1R protein levels showed a similar pattern, i.e., a 2-fold increase in the aortas of VECAD-CYP4A2-transduced rats as compared to control, which was prevented by daily treatment with HET0016 (Figure 3E). Importantly, the expression of the AT2R was not affected by CYP4A2 overexpression or HET0016 treatment; levels of AT2R protein showed similar values in all experimental groups (Figure 3F).

Inhibition of ACE prevents and reverses 20-HETE-mediated hypertension

The possible involvement of the RAS in 20-HETE-mediated hypertension was further examined using lisinopril treatment prior to injection with lenti-VECAD-CYP4A2 and after the lenti-VECAD-CYP4A2-mediated increase in systolic blood pressure was established. As seen in Figure 4A, a bolus lenti-VECAD-CYP4A2 injection rapidly increased blood pressure within 3 days from 101±2 to 139±1 mmHg, reaching 150±2 mmHg at day 10 post-infection. At day 10, administration of lisinopril in the drinking water lowered blood pressure to control levels; however, blood pressure returned to its high levels upon cessation of lisinopril treatment (Day 19) and reached 163±3 mmHg at day 37 post-infection. As previously shown, injection of lenti-VECAD-GFP to age-matched normotensive rats did not affect blood pressure (Figure 4A).

Another group of rats was given lisinopril 3 days before lenti-VECAD-CYP4A2 injection. In this group, blood pressure did not increase in response to the injection with lenti-VECAD-CYP4A2 as long as lisinopril was given in the drinking water. However, upon cessation of lisinopril treatment (day 10 post-infection), blood pressure increased rapidly to 154±4 and 156±2 mmHg at day 17 and 24 of the experiment, respectively (Figure 4B). To further demonstrate the 20-HETE dependency of blood pressure elevation in lenti-VECAD-CYP4A2-transduced rats, treatment with the 20-HETE antagonist, 20-HEDE, was initiated in this group at day 24 of the experiment. Blood pressure gradually decreased, reaching levels of 116±2 and 114±2 mmHg at day 33 and 37 of the experiment (Figure 4B).

20-HETE antagonist attenuates 20-HETE vascular actions and CYP4A2-induced increase in ACE and AT1 expression

CYP4A2 expression increased by 40% in aortas and renal interlobar arteries from lenti-VECAD-CYP4A2-transduced rats as compared to those from control lenti-VECAD-GFP-transduced rats. CYP4A2 expression in arteries from lenti-VECAD-CYP4A2-transduced rats was not altered by treatment with 20-HEDE (Figure 5A and B). The level of 20-HETE in renal interlobar arteries showed a similar pattern; it was higher in arteries from lenti-

VECAD-CYP4A2-transduced rats as compared to arteries from lenti-VECAD-GFP-transduced rats (48.2±6.1 vs 34.8±3.0 ng/mg, n=4, $p<0.05$). CYP4A2 transduction had no significant effect on epoxygenase activity in renal interlobar arteries (Levels of EETs+DHTs in arteries from lenti-VECAD-GFP and Lenti-VECAD-CYP4A2 transduced rats were 75.27±9.94 and 69.93±13.95 ng/mg, respectively). Plasma levels of 20-HETE or EETs were not altered by CYP4A2 transduction (Figure S2). 20-HETE has been shown to sensitize renal arteries to constrictor stimuli such as phenylephrine[26]. As seen in Figure 5C, renal interlobar arteries from lenti-VECAD-CYP4A2-transduced rats were more sensitive to phenylephrine than arteries from lenti-VECAD-GFP-transduced rats as denoted by the EC_{50} to phenylephrine (0.34±0.10 vs 1.03±0.03 μM, n=4, $p<0.05$). Treatment with 20-HEDE right-shifted the phenylephrine concentration-response curve, increasing ($P<0.05$) the EC_{50} to 0.58±0.02 μM (n=4). The relaxation response of interlobar arteries to acetylcholine was significantly diminished in the arteries from lenti-VECAD-CYP4A2-transduced rats as compared to arteries from lenti-VECAD-GFP-transduced rats. Moreover, the relaxing response to acetylcholine was partially restored in arteries from lenti-VECAD-CYP4A2-transduced and 20-HEDE-treated rats (Figure 5D). Arteries from control rats treated with lisinopril had the same responses as arteries from control untreated rats. Addition of either lisinopril or losartan ex vivo attenuated 20-HETE-mediated impairment of acethylcholine-induced relaxation (Figure S3).

ACE (Figure 6A and B) and AT1R (Figure 6C and D) expression were higher in aortas and renal interlobar arteries from lenti-VECAD-CYP4A2-transduced rats as compared to those from control lenti-VECAD-GFP-transduced rats. Moreover, treatment with 20-HEDE significantly reduced the expression of ACE and AT1R. The vascular expression of AT2R was not different among the four groups (data not shown).

DISCUSSION

The present study is the first to suggest that 20-HETE mediates its pro-hypertensive actions through the activation of the RAS, primarily by inducing ACE, leading to Ang II-dependent hypertension.

The expression of CYP4 enzymes has been linked to the pathogenesis of hypertension in experimental animal models and humans through their catalytic activity as 20-HETE biosynthetic enzymes. The pro-hypertensive actions of 20-HETE are believed to be the result of its effect on vascular function. 20-HETE is a prominent eicosanoid in the microcirculation where it promotes constrictor mechanisms by sensitizing the smooth muscle to constrictor stimuli [1] and by limiting endothelial-dependent vasorelaxation [2, 4, 5], most likely through interference with NO bioavailability via eNOS uncoupling [3] and stimulation of superoxide production [27]. Consequently, increased expression of vascular 20-HETE leads to hypertension whereas inhibition of vascular 20-HETE in experimental models of hypertension reduces blood pressure. Experimental models of hypertension that show increased vascular 20-HETE production include the spontaneously hypertensive rat [28, 29] and the androgen-induced hypertensive rats and mice [5, 10, 11]. Both models are Ang II-dependent [30, 31]; however, they present complex models for investigating the possible relationship between Ang II and 20-HETE in the control of blood pressure. Animal models that overexpress 20-HETE-producing CYP4 enzymes [6, 25] are hypertensive and, thus, provide good models for delineating the mechanisms contributing to 20-HETE-mediated hypertension. The observation that 20-HETE stimulates ACE expression in vitro raised the possibility that the RAS is part of 20-HETE pro-hypertensive actions.

The first indication of RAS involvement in 20-HETE-mediated hypertension came from the observation that losartan decreased blood pressure in the CYP4A2-transduced rats. As

previously reported [25] and substantiated in this study, this is an animal model that clearly shows 20-HETE-dependent hypertension based on the ability of the 20-HETE synthesis inhibitor or 20-HETE antagonist to prevent or reverse the hypertension, respectively. Once losartan was withdrawn, blood pressure rapidly returned to its high levels. Our interpretation of this data may be complicated by the involvement of other components of the RAS evoked by the development of hypertension. Thus, the effect of losartan could be independent of the initiating factor, i.e., 20-HETE. However, the demonstration that injection of lenti-VECAD-CYP4A2 in the presence of lisinopril failed to increase blood pressure and that blood pressure remained low as long as lisinopril was present in the drinking water clearly suggested that RAS activation contributed to 20-HETE-dependent hypertension. That the RAS system may be activated by 20-HETE is further supported by the following findings: 1) a 2–3-fold increase in vascular ACE and AT1R mRNA and protein expression in the CYP4A2-transduced rats compared to GFP-transduced rats; 2) suppression of elevated expression of ACE and AT1R in the CYP4A2-transduced by inhibition of 20-HETE synthesis or blockade of 20-HETE action; and importantly 3) an increase in circulatory and vascular tissue Ang II in CYP4A2-transduced rats that is reduced by treatment with 20-HETE inhibitor. In addition, the observation that treatment of normotensive control rats with HET0016 decreased the basal expression levels of ACE suggests that 20-HETE may serve as an endogenous regulator of RAS expression through its action on ACE.

Since 20-HETE's pro-hypertensive properties stem from its actions in the vasculature and since CYP4A2 overexpression was targeted to the vasculature, it is possible that the involvement of the RAS in 20-HETE-dependent hypertension is primarily localized to the vasculature. The fact that Ang II levels in the kidney, as opposed to those in the vasculature, were not significantly elevated supports this notion. Evidence for the presence of RAS in the vascular wall including renin, AGT, ACE and AT receptors and the ability of vascular cells to produce Ang II has been described [32–]34. The observation that in vitro 20-HETE potently and specifically induced ACE expression without significantly altering the expression of AGT, AT1R or AT2R suggests that induction of endothelial ACE by 20-HETE may be a key step in the development of hypertension in this model which is driven by endothelial-specific overexpression of the CYP4A2. ACE has been shown to be highly expressed in the vascular endothelium while the media and the adventitia show little or low levels of ACE expression 35. Kessler et al 36 have expressed ACE in the vascular endothelium of ACE null mice and demonstrated that endothelial ACE is essential for maintaining BP, while Cole et al 37 showed that deletion of endothelial ACE does not alter blood pressure. Our study indicates that under conditions of increased 20-HETE synthesis, vascular ACE expression is increased. More importantly, inhibition of 20-HETE synthesis or blockade of 20-HETE action prevented and reversed the increased expression of ACE in the VECAD-CYP4A2-transduced rats, strongly implicating 20-HETE as a modulator of ACE expression. Our data also indicate that the increase in 20-HETE in VECAD-CYP4A2-transduced rats is associated with an increase in vascular AT1R expression. Such an increase could be the consequence of increased Ang II levels. The mechanism by which 20-HETE induces ACE expression is unknown. We have shown that the 20-HETE effect on endothelial cell NO production and NF-kB activation is mediated through tyrosine kinase/EGFR- and MAPK (ERK 42/44)-dependent IKK activation [38]; preliminary data suggested that induction of ACE by 20-HETE in endothelial cells is also IKK-mediated (Cheng et al, unpublished data).

In summary, It is possible that the induction of ACE within the vascular endothelium sets in motion an increase in vascular Ang II and that such increases contribute, possibly through Ang II-AT1R interactions, to the hypertension brought about by increasing vascular 20-HETE levels. The ability of a 20-HETE inhibitor or antagonist to prevent/reverse hypertension, the capacity of an AT1R blocker to reduce BP, and the ability of ACE inhibitor to prevent hypertension in this model of 20-HETE-dependent hypertension support

this possibility. However, the spatial and temporal relationships between 20-HETE and ACE induction and the causative role of 20-HETE-induced endothelial ACE expression in hypertension need to be further explored.

Perspective

The biology of 20-HETE in the vasculature parallels that of Ang II. Like Ang II, 20-HETE is a vasoconstrictor and a mitogen. Actions of Ang II in vascular cells, including superoxide/ROS stimulation, NF-κB activation and induction of inflammatory molecules, are also shared by 20-HETE. Such similarities raised the question of whether 20-HETE serves as the mediator of Ang II actions. The present study demonstrates that 20-HETE is a potent inducer of ACE and that an ACE inhibitor or AT1R blocker prevents or reverses the hypertension brought about by increased vascular (endothelial) expression of CYP4A2, which is a model of 20-HETE-dependent hypertension. These studies set forth a novel paradigm and an attractive hypothesis where excessive production of 20-HETE within the vasculature, such as in androgen-induced hypertension, leads to hypertension via mechanisms that include the induction of endothelial ACE, thus perpetuating an increase in vascular Ang II which, in turn and together with 20-HETE, promotes vascular dysfunction. This study is potentially of great significance as it brings into being a new face to an old paradigm and suggests a feed forward amplification of vascular dysfunction and hypertension brought about by the induction of ACE by 20-HETE. The importance of elucidating and understanding the mechanisms underlying 20-HETE pro-hypertensive actions is underscored by recent reports linking CYP4 polymorphism and 20-HETE levels to hypertension in humans.

Supplementary Material

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Acknowledgments

Source of Funding: National Institutes of Health: HL34300 (MLS), DK068134 and HL55601 (NGA), F30 HL097402 (CCW), and GM31278 (JRF), and by the Robert A. Welch Foundation (JRF).

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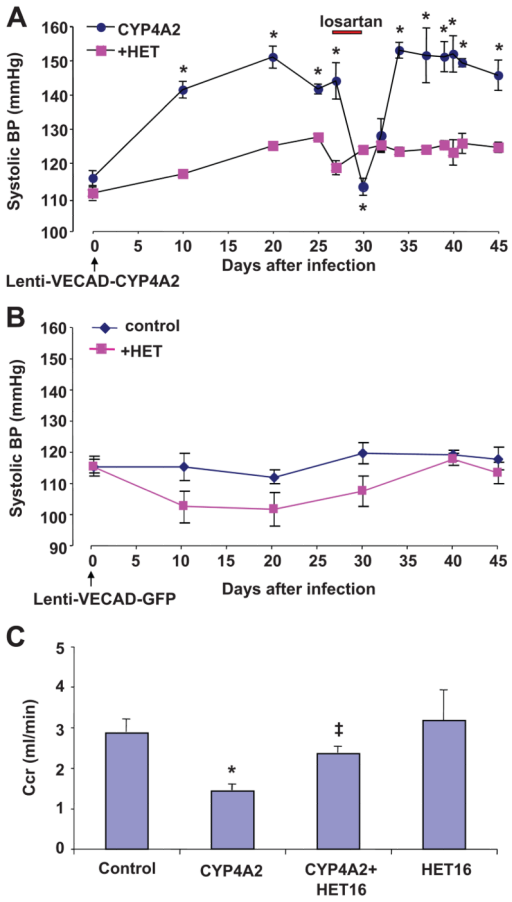

Figure 1.

Effect of AT1R blockade and 20-HETE inhibition on blood pressure in VECAD-CYP4A2-transduced rats. Rats were injected with Lenti-VECAD-CYP4A2 **(A)** or the Lenti-VECAD-GFP **(B)** and treated with and without HET0016 (HET; 10mg/kg/day). At day 27, Losartan (100 mg/kg) was added to the drinking water of Lenti-VECAD-CYP4A2-injected rats (A) for 3 days (n=4, *p<0.05 vs day 0, ‡p<0.05 vs day 30). **C)** Creatinine clearance (Ccr) in lenti-VECAD-GFP transduced and lenti-VECAD-CYP4A2 transduced rats with and without HET0016 was measured at the end of the experiment (n=4; *p<0.01 vs control; ‡p<0.01 vs CYP4A2).

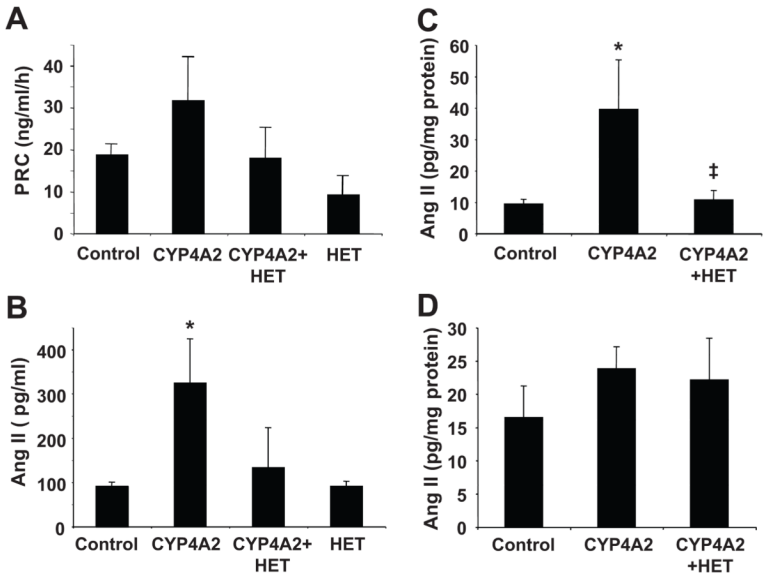

Figure 2.

(A) Plasma renin concentration and levels of Ang II in plasma **(B),** aorta **(C)** and kidney **(D)** in VECAD-GFP-transduced rats (control) and VECAD-CYP4A2-transduced rats (CYP4A2) treated and untreated with HET0016 (HET). Blood was taken at the end of the experiment (n=4, *$p<0.05$ vs control; ‡$p<0.05$ vs CYP4A2).

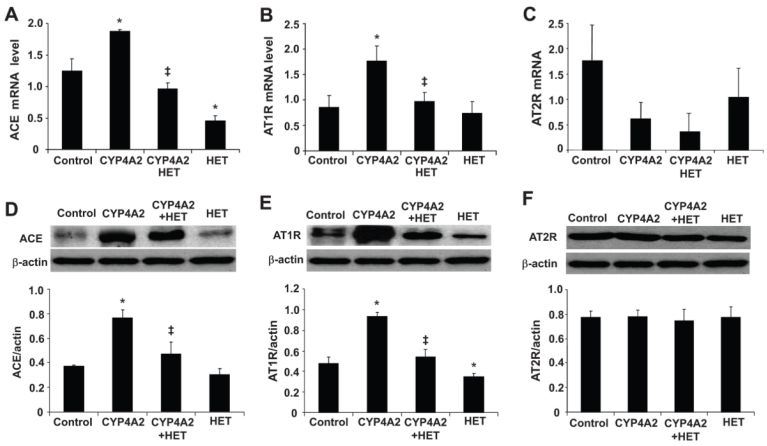

Figure 3.

Real-time PCR and western blot analyses of ACE (A, D), AT1R (B, E) and AT2R (C, F) in aorta from control (VECAD-GFP-transduced rats) and VECAD-CYP4A2-transduced rats (CYP4A2) treated and untreated with HET0016 (HET) (n=4, *p<0.05 vs control; ‡p<0.05 vs CYP4A2).

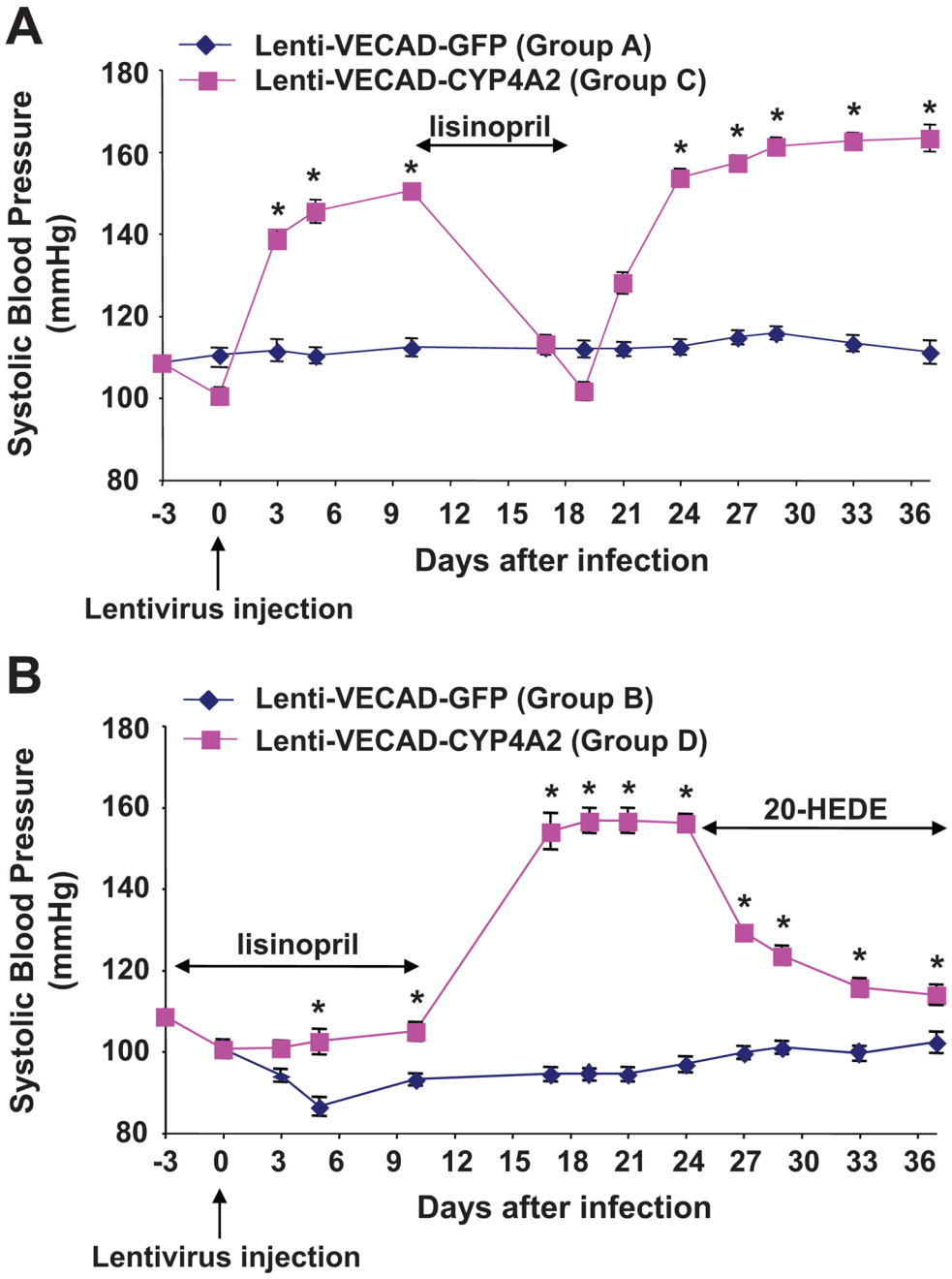

Figure 4.

Effect of lisinopril and 20-HEDE on blood pressure in rats transduced with lenti-VECAD-GFP (Group A and B) or lenti-VECAD-CYP4A2 (Group C and D). Lisinopril was given in the drinking water at day 10 post-lentivirus injection **(A)** or 3 days prior to and the first 10 days after lentivirus injection **(B)**. Group B was treated with lisinopril for the entire duration. Group D was given 20-HEDE daily starting at day 24 (n=4; *p<0.05 vs corresponding group A or B).

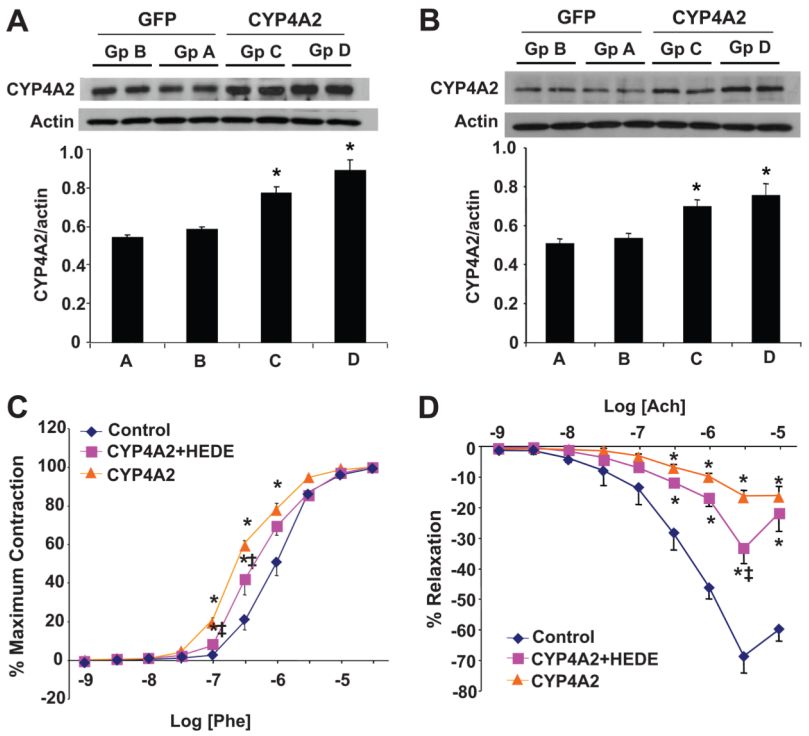

Figure 5.

Western blot of CYP4A2 in aortas **(A)** and renal interlobar arteries **(B)** from rats transduced with lenti-VECAD-GFP treated with and without lisinopril (Gp B and A, respectively) and lenti-VECAD-CYP4A2 treated with and without 20-HEDE (Gp D and C, respectively) (n=4, *p<0.05 vs control). The contractile response to phenylephrine **(C)** and relaxing response to acetylcholine **(D)** in renal interlobar arteries from rats transduced with lenti-VECAD-GFP (Control) and lenti-VECAD-CYP4A2 (CYP4A2) treated with and without 20-HEDE (CYP4A2+HEDE) during the last 10 days of the experiment (n=4, *p<0.05 vs control; ‡p<0.05 vs CYP4A2).

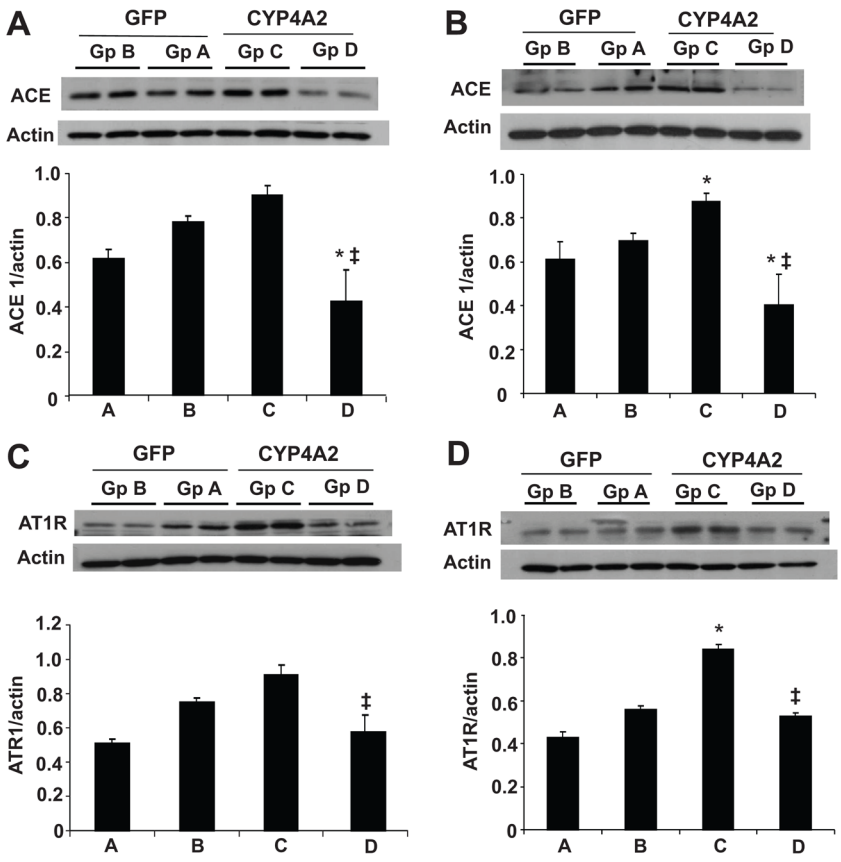

Figure 6.

Western blot of ACE (A, B) and AT1R (C, D) in aortas and renal interlobar arteries from rats transduced with the lenti-VECAD- GFP treated with and without lisinopril (Gp B and A) or lenti-VECAD-CYP4A2 treated with and without 20-HEDE (Gp D and C, respectively) as in Figure 6 (n=4, *p<0.05 vs. control, #p<0.05 vs. CYP4A2).