EP1 Disruption Attenuates End-Organ Damage in a Mouse Model of Hypertension

Christina S. Bartlett, Kelli L. Boyd, Raymond C. Harris, Roy Zent, Richard M. Breyer

Abstract—Prostaglandin E₂ is a major prostanoid found in the kidney and vasculature contributing to the regulation of blood pressure. The prostaglandin E₂ receptor EP1 has been shown to contribute to hypertension by mediating angiotensin II-dependent vasoconstriction, although its precise role is incompletely characterized. Disruption of the EP1 receptor in C57BL/6J mice reduced the incidence of mortality during severe hypertension induced by uninephrectomy, deoxycorticosterone acetate, and angiotensin II. Mortality was dependent on all components of the model. Death was a result of aortic aneurysm rupture or occurred after development of anasarca, each of which was reduced in EP1−/− mice. Mean arterial pressure was increased in treated EP1+/+ and EP1−/− mice; however, this elevation was significantly lower in EP1−/− mice. Blood pressure reduction via administration of hydralazine phenocopied EP1−/− mice. Thus, reduction in blood pressure by disruption of EP1 reduced incidence of mortality and decreased organ damage, suggesting that EP1 receptor blockade may be a viable target for antihypertensive therapy. (Hypertension. 2012;60:1184-1191.)

Key Words: prostaglandins • hypertension • aorta • aneurysm • edema

Hypertension is a major risk factor for cardiovascular diseases, increasing the risk of stroke, myocardial infarction, arterial aneurysms, and heart failure. Approximately 30% of adults in the United States have hypertension, and the incidence of cardiovascular diseases remains greater in hypertensive patients than in normotensive patients, highlighting the need for novel therapeutic agents.

Prostaglandin E₂ (PGE₂) is a major prostanoid found in the mouse kidney and vasculature contributing to the regulation of blood pressure, where it can exert either vasopressor or vasodepressor effects. Four PGE₂ receptors (EP1 through EP4) mediate these effects, with the EP1 and EP3 receptors primarily mediating the pressor response of PGE₂, whereas the EP2 and EP4 receptors mediate the depressor response.

Each PGE₂ receptor has distinct tissue localization and elicits characteristic signal transduction pathways. EP1 couples to Gₛ proteins, resulting in mobilization of intracellular calcium and stimulation of phosphoinositide turnover activating protein kinase C. The EP2 and EP4 receptors couple to Gₛ proteins, which increase intracellular cAMP. The EP3 receptor couples to G₁ proteins, decreasing intracellular cAMP (for reviews, see Coleman et al and Sugimoto et al). Receptors couple to alternate signal transduction pathways as well, including arrestin-mediated signaling pathways.

Systemic infusion of PGE₂ results in a vasodepressor response; primarily through EP2 activation. In the absence of EP2, a PGE₂ pressor response is unmasked, mediated by the EP3 receptor. Agonist-induced EP3 tachyphylaxis in the background of EP2−/− mice uncovers a depressor action of EP4. EP1 does not appear to play a significant role in the blood pressure effects of systemically administered PGE₂; however, it does contribute to hypertension. Genetic disruption of the EP1 receptor in mice has been shown to decrease blood pressure, particularly when mice are fed a low-sodium diet. Furthermore, EP1−/− mice have blunted pressor responses to both acute and chronic angiotensin II (Ang II) administration. In isolated vessel preparations, pretreatment with the EP1 selective antagonist SC51322 reduced Ang II-mediated vasoconstriction. Treatment of spontaneously hypertensive rats with SC51322 significantly reduces blood pressure, indicating that blockade of the EP1 receptor may be a target for the treatment of hypertension.

EP1 blockade has been shown to affect renal function positively in stroke-prone spontaneously hypertensive rats, as well as cerebrovascular dysfunction induced by Ang II, implicating the EP1 receptor in hypertension and resultant end-organ damage. This has yet to be investigated in detail and in the context of other organ damage. Therefore, EP1+/+ and EP1−/− mice were studied in a model of severe hypertension.

Methods

Complete methods can be found in the expanded Methods section in the online-only Data Supplement.
Results

EP1−/− Mice Were Protected Against Nphx/DOCA-NaCl/Ang II Mortality

We used a model of severe hypertension to investigate the contribution of the EP1 receptor in hypertensive end-organ damage. Unexpected mortality was observed after implantation of the Ang II osmotic pump (Figure 1A). Of 58 EP1+/+ mice, 60% died within 14 days. EP1−/− mice were significantly protected against mortality; of 35 EP1−/− mice only 24% died (P=0.0044). Modified protocols omitting 1 of the 3 components (Nphx, DOCA-NaCl, or Ang II) demonstrated that all components of the model played an essential role in causing mortality (Figure 1B, Nphx/DOCA-NaCl/Ang II versus Nphx/DOCA-NaCl, Nphx/Ang II, or DOCA-NaCl/Ang II; N=10 per group; P=0.011, <0.005, or <0.005, respectively). Modified protocols omitting 1 of the 3 components (Nphx, DOCA-NaCl, or Ang II) demonstrated that all components of the model played an essential role in causing mortality (Figure 1B, Nphx/DOCA-NaCl/Ang II versus Nphx/DOCA-NaCl, Nphx/Ang II, or DOCA-NaCl/Ang II; N=10 per group; P=0.011, <0.005, or <0.005, respectively).

EP1−/− Mice Had Reduced Aortic Aneurysm Rupture but Comparable Aortic Histopathology

Postmortem analysis of EP1+/+ and EP1−/− mice in the full model indicated that a significant portion of the mice died as a result of rupture of the aorta. Aneurysms and dissections were observed in both the thoracic and abdominal aortas. A total of 37% of EP1+/+ and 13% of EP1−/− mice died because of aortic rupture. Aneurysms were present in 67% of EP1+/+ mice and 40% of EP1−/− mice. Aneurysm severity was scored at death on a scale of 0 to 5 (Figure 2A). A reduction in aneurysm severity was observed in EP1−/− mice compared with EP1+/+ mice (Figure 2B; P=0.049). Hematoxylin and eosin staining revealed aneurysms, and dissections in the wall of the thoracic and abdominal aortas were accompanied by inflammation in both EP1+/+ and EP1−/− mice (Figure 3A through 3E; P=0.3975). Analysis of aortic sections from both thoracic and abdominal lesions of each genotype demonstrated macrophage, and neutrophils were most abundant, with no differences observed between the genotypes in any immune cell component (Figure 3F). Cyclooxygenase 2 (COX-2) has been shown to play a significant role in aortic aneurysm formation and macrophage infiltration.21,22 COX-2 mRNA was elevated although this difference was modest (EP1+/+, 26.6±0.39 g; EP1−/−, 24.9±0.68 g; P=0.024). Body weight of EP1−/− mice developed aneurysms as a result of the Nphx/DOCA-NaCl/Ang II model. Fisher exact test, EP1+/+ vs EP1−/− severity <3 vs ≥3. Ang II indicates angiotensin II; P=0.049.

Figure 1. Survival. A, Survival of EP1+/+ and EP1−/− mice. EP1+/+ mice experience a high mortality rate after implantation of the angiotensin II (Ang II)-minipump (60%, N=58), which is significantly reduced in EP1−/− mice (25%, N=35). Kaplan-Meier survival curves for each genotype are plotted. **P=0.004. B, Survival of EP1+/+ modified protocol groups. Survival curves were plotted indicating reduced mortality in all modified protocol groups. Nphx/DOCA-NaCl/Ang II (data replotted from A) vs Nphx/DOCA-NaCl, Nphx/Ang II, or DOCA-NaCl/Ang II; P=0.011, <0.005, or <0.005.

Figure 2. Aortic aneurysm formation in EP1+/+ and EP1−/− mice. A, Nphx/DOCA-NaCl/Ang II treatment resulted in formation of aneurysms and dissections in the thoracic and abdominal aortas. Aortas were scored visually on necropsy, and representative images are shown. B, Postmortem examination revealed 18 of 27 EP1+/+ mice and 6 of 15 EP1−/− mice developed aneurysms as a result of the Nphx/DOCA-NaCl/Ang II model. Fisher exact test, EP1+/+ vs EP1−/− severity <3 vs ≥3. Ang II indicates angiotensin II; P=0.049.
was unchanged over the course of the study. EP1+/+ mice had a significantly greater fraction water weight compared with EP1−/− mice (Figure 4B; \( P = 0.0138 \)), and aneurysm incidence was lower in mice which developed anasarca compared with mice without anasarca (33% versus 76%). Anasarca is commonly a result of liver failure, nephrotic syndrome, or heart failure. Plasma alanine transaminase activity was analyzed as a marker of liver function in Nphx/DOCA-NaCl/Ang II-treated EP1+/+ mice. Alanine transaminase was not elevated above baseline values nor did it correlate with body weight (data not shown).

Modest Renal Injury Was Induced in EP1+/+ and EP1−/− Mice

The Nphx/DOCA-NaCl/Ang II model was initially developed to induce hypertensive renal damage on the C57BL/6 background. To quantify renal damage in the EP1+/+ and EP1−/− mice, we monitored urinary albumin excretion, serum urea nitrogen, renal histopathology, and biomarkers of acute kidney injury, neutrophil gelatinase-associated lipocalin, and kidney injury molecule 1 mRNA expression (Figure 5).

Albumin creatinine ratio and serum urea nitrogen were elevated,
but no significant differences were observed between genotypes (Figure 5A and 5B). Renal histopathology showed modest hypertensive renal damage compared with the contralateral kidney removed at time of uninephrectomy (Figure 5C). Dilated tubules with moderate glomerular sclerosis and tubulointerstitial fibrosis were observed. Significant increases in neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 mRNA expression in the kidney of EP1+/+ and EP1−/− mice after treatment with Nphx/DOCA-NaCl/Ang II treatment compared with baseline (EP1+/+, P=0.0029; EP1−/−, P=0.0003; Kim-1; EP1+/+, P=0.007; EP1−/−, P=0.0002), although no significant differences between EP1+/+ and EP1−/− mRNA levels were observed (Ngal, P=0.8609; Kim-1, P=0.4931; N=3–7).

Hypertension Was Less Severe in EP1−/− Mice Than in EP1+/+ Mice

To determine the effect of disruption of the EP1 receptor on blood pressure in Nphx/DOCA-NaCl/Ang II-treated animals, intracarotid blood pressure was determined in EP1+/+ and EP1−/− mice 2 days after Ang II administration (Figure 6). Mean arterial pressure (MAP) was significantly higher in EP1+/+ than EP1−/− mice after treatment with Nphx/DOCA-NaCl/Ang II showed no significant difference between the genotypes (EP1+/+: 198.9±9.6; N=21, EP1−/−: 190.0±4.3; N=17; P=0.461).

Cardiac Function Is Reduced in EP1+/+ and EP1−/− Mice

No structural differences in the heart were observed by echocardiography between EP1+/+ and EP1−/− mice at baseline. A modest increase in ejection fraction and fractional shortening was observed in EP1−/− mice at baseline. At 5 days after Ang II administration, EP1+/+ had increased left ventricular posterior wall and interventricular septum diastolic diameters. EP1−/− mice had increased interventricular septum diastolic diameters, although no significant change in left ventricular posterior wall was observed. EP1+/+ and EP1−/− mice displayed increased left ventricular interior diameter, although no differences were observed between genotypes. Cardiac function was significantly reduced in both genotypes on treatment with Nphx/DOCA-NaCl/Ang II, as demonstrated by decreased fractional shortening and ejection fraction (Table). Additionally, heart weights of EP1+/+ and EP1−/− mice after treatment with Nphx/DOCA-NaCl/Ang II showed no significant difference between the genotypes (EP1+/+: 198.9±9.6; N=21, EP1−/−: 190.0±4.3; N=17; P=0.461).

**Table. Echocardiography Data at Baseline and 5 Days After Ang II Treatment**

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*P<0.05, Baseline vs Nphx/DOCA-NaCl/Ang II.
†P<0.05, EP1+/+ vs EP1−/−.
Discussion

Disruption of the EP1 receptor affords substantial protection in the Nphx/DOCA-NaCl/Ang II-evoked hypertension. The incidence of mortality was significantly decreased and appeared to result from reduction in MAP. Mortality was a result of ruptured aortic aneurysm or occurred after developing anasarca. The results presented here are consistent with previous studies demonstrating that disruption of the rise in MAP in response to Ang II, and furthermore reveal the protective effect disruption that the EP1 receptor has on end-organ damage.

There are several limitations to these studies that deserve mention. First, high mortality observed in EP1+/+ mice confounds the analysis of measurements taken after implantation of Ang II, such as cardiac and renal function, because the analyses are only performed on surviving mice. Second, there was a modest (<10%), although statistically significant, difference in body weight observed between EP1+/+ and EP1−/− mice. Although the dosage of Ang II was adjusted by weight, the dosage of the DOCA pellet was not. However, one would predict the genotype receiving the greater dose/weight (EP1−/−) would have the worse phenotype, and this is opposite of what we observed. Lastly, EP1−/− mice were observed to have lower blood pressure than EP1+/+ mice after treatment with the Nphx/DOCA-NaCl/Ang II model. This is consistent with our data published previously, suggesting that EP1 mediates part of Ang II-induced hypertension. In this model we measured MAP at baseline or after treatment with all 3 model components; it is possible that EP1 also contributes to hypertension induced by Nphx or DOCA-NaCl as well.

Current models of aortic aneurysm include a combination of hyperlipidemic mice or high-fat diet with modulation of the renin-angiotensin-aldosterone axis or aberrant production of extracellular matrix components. Ang II-induced aortic aneurysms are characterized by accumulation of macrophages in the adventia and media, disruption of elastin fibers, expansion of the lumen, thrombus formation, and disordered extracellular matrix deposition. These characteristics were also observed in the Nphx/DOCA-NaCl/Ang II model, although no significant differences in macrophage accumulation, matrix deposition, or COX-2 mRNA expression were detected between the 2 genotypes. It should be noted that the aneurysms and dissections observed in this model occur after acute severe hypertension, and although the pathology appears similar to that observed in human disease, the disease genesis may not be. In humans, development of a true aneurysm is a slowly progressing disease initiating with local inflammation and disruption of the connective tissue matrix and is often associated with atherosclerosis. In contrast, development of false aneurysm or dissection as a result of a tear in the intima can occur more acutely by a sudden large rise in blood pressure or direct injury and may be more representative of the damage induced by the Nphx/DOCA-NaCl/Ang II model. Our data demonstrate that protection observed when EP1 is disrupted is likely because of the prevention of a large rise in blood pressure in EP1−/− mice, including the reduction in MAP in EP1+/+ mice (106.1±5.76 mm Hg; P=0.0004, EP1−/− #P=0.0295) but had no significant effect on EP1−/− mice (Figure 7A, 99.47±4.82 mm Hg; P=0.0423). Anasarca was not observed in hydralazine-treated EP1−/− mice. Anasarca was not observed in hydralazine-treated EP1−/− mice (body weight at 5 days post-Ang II: 21.40±0.65 g) as compared with untreated EP1−/− mice (body weight at 5 days post-Ang II: 32.28±1.59 g; P=0.0295).

Figure 6. Mean Arterial Pressure (MAP) in EP1+/+ and EP1−/− mice. Intracarotid blood pressure was measured in EP1+/+ and EP1−/− mice 2 days post-angiotensin II administration under ketamine and inactin anesthesia. MAP was elevated in both groups compared with baseline (EP1+/+ *P=0.0004, EP1−/− #P=0.0423) but was significantly lower in EP1−/− compared with EP1+/+ 2 days post-angiotensin II (##P=0.0295).
pressure, because treatment with hydralazine phenocopied EP1−/− mice. This does not eliminate the possibility that EP1 receptors might also provide protection directly at the target tissue.

Data exist suggesting a role for prostaglandins, in particular PGE2, in aortic aneurysm formation. COX-2 initiates the production of prostaglandins, and its expression is induced by infusion of Ang II in the smooth muscle of the aorta surrounding aneurysms.21 Furthermore, either selective inhibition of COX-2 or genetic deletion of COX-2 significantly reduced aortic aneurysm formation and macrophage infiltration.21,22 Deletion of microsomal PGE synthase 1, which transforms the product of COX-2 metabolism into PGE2, has also been demonstrated to reduce aortic aneurysm formation and oxidative stress in low density lipoprotein receptor null mice with an Ang II infusion,27 suggesting that PGE2 plays an important role in development of aneurysms and the EP receptors may be viable targets for treatment of aneurysm progression.

Previous reports of the role of EP1 in renal injury are contradictory. In spontaneously hypertensive rats, treatment with an EP1 antagonist reduced proteinuria and tubulointerstitial damage,18 whereas in anti-glomerular basement membrane nephrotoxic serum nephritis genetic deletion of EP1−/− in mice resulted in enhanced mesangial expansion and tubular dilation and increased serum urea nitrogen and serum creatinine.28 In our studies, modest hypertensive renal damage was observed, although no significant differences in renal function were detected between genotypes. However, our interpretation was confounded by the differential mortalities in EP1+/+ and EP1−/− mice, potentially biasing our results. Examination of renal histopathology at time points before significant mortality failed to detect any severe renal damage or differences between the genotypes. This suggests that the role of EP1 in renal damage is highly context dependent.

Anasarca, or extreme generalized edema, can occur in many disease settings. It is commonly a result of liver failure, nephrotic syndrome, or heart failure.23,24 In our
Nphs/DOCA-NaCl/Ang II model, a subset of EP1+/- mice developed severe anasarca before mortality, whereas EP1-/- mice were protected. The EP1 receptor has been shown previously to be natriuretic. 10 With this paradigm, one might predict that EP1-/- mice would retain more salt and water; however, in our results we demonstrate that EP1+/- mice gain excessive fluid volume that is not observed in EP1-/- mice. This contradiction leads us to conclude that alterations in kidney function by disruption of EP1 do not play a dominant role in the development of the observed edema. Additionally, cardiac function was reduced to similar degrees in EP1+/- and EP1-/- mice. Edema was prevented by treatment with hydralazine, suggesting elevation in blood pressure was responsible for development of edema. We hypothesize that hypertension induced by DOCA-NaCl and Ang II results in volume loading and enhanced vasoconstriction, which places excessive stress on the vascular wall leading to enhanced permeability, resulting in edema and susceptibility to dissections and rupture. Future experiments will be required to identify whether vascular permeability differences are observed between EP1+/- and EP1-/- mice.

Perspectives

The EP1 receptor plays an important role in the development of hypertensive damage. In the Nphs/DOCA-NaCl/Ang II model, disruption of EP1 results in increased survival, lessened aneurysm severity, and the absence of anasarca. This effect is a result of a reduced rise in blood pressure observed in EP1-/- mice and suggests the EP1 receptor may be a viable pharmacological target for the treatment of hypertension and subsequent organ damage. Furthermore, the Nphs/DOCA-NaCl/Ang II model may prove to be a useful tool for studying the pathology of aortic aneurysm and dissection formation in a setting of acute severe hypertension.

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Disclosures

None.

References


### Novelty and Significance

**What Is New?**
- Describes new model of aortic aneurysm
- Anasarca is observed
- Disruption of the EP1 receptor improves the outcome of each pathology and overall survival

**What Is Relevant?**
- Studies shed light on the role of EP1 in the pathophysiology of hypertension
- Identify a therapeutic target for hypertension and its sequelae

**Summary**

The Nphx/DOCA-NaCl/Ang II model induces hypertension, aortic aneurysm formation, and anasarca. Disruption of the EP1 receptor reduced blood pressure in this model, leading to reduced incidence of mortality and decreased organ damage.
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In the Hypertension article by Bartlett et al (Bartlett CS, Boyd KL, Harris RC, Zent R, Breyer RM. EP1 Disruption Attenuates End-Organ Damage in a Mouse Model of Hypertension. Hypertension. 2012;60:1184–1191), a correction was needed.

An affiliation was erroneously omitted. The affiliation is as follows: VA Tennessee Valley Healthcare System, Nashville, TN (R.C.H., R.Z., R.M.B.).

The authors apologize for the omission.

This correction has been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/60/5/1184.full.
Expanded Methods

Animal procedures
The hypertension model was carried out as described by Kirchhoff and co-workers \(^1\). Ten-to-16 week old male C57BL/6J (EP1+/+; Jackson Labs, USA, N = 58) and EP1-/- mice (N = 35) backcrossed at least ten generations onto C57BL/6J were uninephrectomized (Nphx) under ketamine/xylazine (100 mg/kg i.p. and 15 mg/kg i.p.) anesthesia. Buprenorphine (0.1 mg/kg s.q.) was administered post Nphx as an analgesic. Two weeks later, a subcutaneous 50 mg deoxycorticosterone acetate (DOCA) pellet (Innovative Research of America, USA) was implanted under isoflurane anesthesia (2 %) and drinking water supplemented with 1 % NaCl. After an additional week, a subcutaneous osmotic mini-pump was implanted under isoflurane anesthesia (2 %, Alzet model 1002; Durect Corporation, USA) delivering 1.5 ng angiotensin II (Calbiochem, USA) per minute per gram body weight. Mice were followed for two more weeks when tissues were collected for histology. Additional studies eliminating one of the three elements from the protocol where performed, while keeping the time frame between surgeries consistent (N = 10 mice per group). Animals were maintained in an AAALAC accredited rodent facility in individually ventilated microisolator cages on a 12:12 light dark cycle. All procedures were done in accordance with the policies of the Institutional Animal Care and Use Committee at Vanderbilt University.

Examination of aortic aneurysm and dissection
Upon necropsy aortic aneurysms and dissections were observed in both the thoracic and abdominal aorta, and severity was scored visually using the following scale adapted from Manning et al., 2002 \(^2\). Type 0: no aneurysm, Type 1: dilated aorta with no thrombus, Type 2: remodeled tissue that frequently contains thrombus, Type 3: a pronounced bulbous form of type 2, Type 4: multiple overlapping aneurysms, or a dissection extending the length of the aorta, Type 5: ruptured aorta. Hematoxylin and eosin and Masson’s Trichrome stains was used on formalin-fixed, paraffin-embedded aorta sections. Immunohistochemistry was performed for macrophage (CD11b and F4/80, Novus Biologicals cat# NB600-1327 and NB600-404), neutrophils (myeloperoxidase, Dako cat# A0398), T-cells (CD3, Santa Cruz cat# sc-1127), and B-cells (CD45R/B220, BD Pharmigen cat# 553084). Inflammation, collagen organization and all immunohistochemistry sections were scored at the site of the lesion in a blinded fashion by a comparative veterinary pathologist.

Fraction water weight determination
Fractional water weight was determined as previously described \(^3\). Eight EP1+/+ and seven EP1-/- mice were sacrificed 5 days post-Ang II administration, their carcasses weighed (wet weight) and incubated at 60°C. Body weight was measured daily until it remained unchanged for 3 days, indicating dry body weight. Fractional water weight was determined using the equation:
Fractional water weight = 1 - (dry weight/wet weight).

Determination of urinary albumin/creatinine ratios
Albumin/creatinine ratios (ACR; expressed as mg albumin/mg creatinine) were measured from 20-200 µL volumes of spot urine using Albuwell M ELISA kit, and urinary creatinine was measured using the Creatinine Companion (Exocell, Philadelphia, USA).
Determination of Blood Urea Nitrogen levels
To assess the renal function, blood urea nitrogen (BUN) was determined using an iSTAT-1 analyzer (Abbott Point of Care Inc., New Jersey, USA). Whole blood was obtained from saphenous vein and immediately assayed utilizing Chem8+ cartridges.

Assessment of Renal Histopathology
Tissues were fixed overnight in 10% neutral buffered formalin. Kidneys were processed routinely, embedded in paraffin, sectioned at 5 microns, stained with H&E or Masson’s Trichrome and evaluated by light microscopy.

Quantitative PCR
Total RNA from kidneys and aortae was isolated using the TRIzol reagent followed by RNA cleanup with a Qiagen RNeasy kit. cDNA was made using the high-capacity cDNA archive kit (Applied Biosystems). Quantitative PCR was performed using Applied Biosystems 7900HT Fast Real-time PCR system with Taqman gene expression assays for neutrophil gelatinase-associated lipocalin (Ngal, Mm01324470_m1), kidney injury molecule-1 (Kim-1, Mm00506686_m1), Cyclooxygenase-2 (COX-2) (Mm00478374_m1), and 18S rRNA (4319413E). Fold difference in mRNA expression is plotted relative to a normal EP1+/+ kidney sample.

Echocardiography
Transthoracic echocardiography was performed on lightly-anesthetized (isoflurane) mice using the VisualSonics VEVO2100 system (30 MHz transducer). Left ventricular posterior wall dimensions (LVPW), intraventricular septum dimensions (IVS), left ventricular interior diameter (LVID), fractional shortening (FS) and ejection fraction (EF) were measured for analysis of cardiac structure and function at baseline, prior to uninephrectomy, and five days post-Ang II administration.

Intracarotid blood pressure measurement
Intracarotid blood pressure was measured under ketamine (25 mg/kg) and inactin (100 mg/kg) anesthesia delivered intraperitoneally. Mice were placed on a thermal pad and a PE-10 catheter was inserted into the left carotid artery. The catheter was connected to a TXD-310 transducer and blood pressure was measured using a Digi-Med BPA 400 (Micromed). Mice were equilibrated 30-60 minutes until stable values were attained. Ten minute blood pressure measurements were collected and average mean arterial pressure (MAP) is plotted.

Antihypertensive Treatment
Blood pressure reduction was achieved by adding hydralazine (200 mg/L) to the 1% NaCl drinking water beginning three days prior to angiotensin II mini-pump implantation. Treatment was continued for the duration of the experiment, or a total of 17 days.

Statistical Analysis
Data are means ± SEM, using GraphPad Prism software (GraphPad Software Inc., USA). Analysis utilized Student’s t test and Fisher’s exact test. Kaplan Meier survival curves were evaluated with the Log-rank (Mantel-COX) test. P < 0.05 was considered statistically significant for all studies.
References


Figure S1. Aortic fibrosis. A. Extracellular matrix deposition was determined using Masson’s Trichrome stain (P = 0.1925). B-E. Masson’s trichrome stained aortae. EP1+/+ aorta (B, D) with rupture displays less collagen organization. EP1-/- aorta (C, E) with an intact aneurysm shows well developed and organized collagen (arrows) surrounding the aneurysm. L = vessel lumen, * = aneurysm.