Androgen-Dependent Hypertension Is Mediated by 20-Hydroxy-5,8,11,14-Eicosatetraenoic Acid–Induced Vascular Dysfunction
Role of Inhibitor of κB Kinase

Cheng-Chia Wu, Jennifer Cheng, Frank Fan Zhang, Katherine H. Gotlinger, Mukul Kelkar, Yilun Zhang, Jawahar L. Jat, John R. Falck, Michal L. Schwartzman

See Editorial Commentary, pp 681–682

Abstract—Increased vascular synthesis of 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) is associated with increased vascular contraction, endothelial dysfunction, and endothelial activation; all are believed to account for 20-HETE prohypertensive properties. We demonstrated previously that the 20-HETE–dependent inhibition of NO production is mediated through inhibitor of κB kinase (IKK), suggesting a cross-talk between 20-HETE–mediated endothelial dysfunction and activation. In this study, we examined the temporal relationship among blood pressure, endothelial dysfunction, and endothelial activation and the role of IKK in the rat model of androgen-driven 20-HETE–mediated hypertension. In Sprague-Dawley rats treated with 5α-dihydrotestosterone, renal vascular 20-HETE levels increased by day 2 of treatment from 17.7 ± 2.4 to 57.7 ± 9.7 ng/mg, whereas blood pressure elevation reached significance by day 3 (132.7 ± 1.7 versus 117.2 ± 0.8 mm Hg). In renal interlobar arteries, when compared with vehicle, 5α-dihydrotestosterone treatment increased the sensitivity to phenylephrine-induced vasoconstriction by 3.5-fold, decreased acetylcholine-induced vasorelaxation, and increased nuclear factor κB activity, all of which were attenuated by treatment with the 20-HETE antagonist, 20-hydroxyeicosa-6(Z),15(Z)-dienoic acid, (20-6,15-HEDE). Cotreatment with parthenolide, an IKK inhibitor, attenuated the androgen-dependent 20-HETE–mediated elevation in blood pressure (from 133.7 ± 3.1 to 109.8 ± 3.0 mm Hg). In addition, parthenolide treatment negated 20-HETE–mediated inhibition of the relaxing response to acetylcholine and 20-HETE–mediated increase in vascular nuclear factor κB activity. These findings suggest that inhibition of IKK attenuates the androgen-dependent 20-HETE–mediated increase in blood pressure by inhibiting both 20-HETE–dependent endothelial activation and dysfunction. (Hypertension. 2011;57:788-794.) ● Online Data Supplement

Key Words: cytochrome P450 ■ 20-HETE ■ IKK ■ NF-κB ■ androgen

Sex differences are observed in many aspects of mammalian cardiovascular physiology and pathology. In particular, hypertension is more common in men than in premenopausal women of the same age.1-2 Epidemiological, clinical, and experimental studies have shown that androgens may be important determinants of sex-specific differences in arterial blood pressure (BP).3,4 Androgens have been hypothesized to modulate BP at the cardiac and the vascular levels.4,5 Sex-specific differences in BP are also observed in various animal models, including male spontaneously hypertensive rats,6-8 Dahl salt-sensitive rats,9 deoxycorticosterone acetate-salt hypertensive rats,10,11 New Zealand genetically hypertensive rats,12 and Cyp4a14/f−/− knockout mice.13 In all, males demonstrated higher BP than females. Treatment of Sprague-Dawley rats with androgen increased BP in both male and female rats.14 Recent studies in our laboratory implicated a role for vascular CYP4A-derived 20-hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) in the development of androgen-induced hypertension and suggested that 20-HETE is a key mediator of androgen-induced vascular resistance and hypertension.14-16

20-HETE is a primary arachidonic acid metabolite in the renal microcirculation that is generated by enzymes of the CYP4 gene family and participates in the regulation of vascular tone via its actions on both vascular smooth muscle and endothelial cells. In vascular smooth muscle cells, 20-HETE inhibits the large conductance Ca2+-activated K+ channel, leading to depolarization and elevation in cytosolic
Vehicle
B
phosphorylation of inhibitor of
synthesis inhibitors or 20-HETE antagonists. 16,21,22 Thus, al20 demonstrated that 20-HETE–mediated endothelial NO
20-HETE attenuates the relaxing responses to acetylcholine
between vascular 20-HETE and NF-
synthase uncoupling is mediated through activation of IKK,
suggesting the existence of a cross-talk between 20-HETE–
synthetic activation by activ-
tion of superoxide production, and activation of a proinflammatory program.15,19,20 In renal microvessels, 20-HETE attenuates the relaxing responses to acetylcholine20; this endothelial dysfunction is reversed by 20-HETE synthesis inhibitors or 20-HETE antagonists.16,21,22 Thus, enhanced production of 20-HETE within the vasculature, as in the spontaneously hypertensive rats,23,24 the androgen-dependent hypertensive rats,16,25 and mice,13 or in CYP4A2-transduced rats,21,22 contributes to hypertension, and inhibition of 20-HETE synthesis or action lowers BP in these models. In humans, increased urinary excretion of 20-HETE has been correlated with hypertension and endothelial dys-
function.26,27 These studies clearly implicated an important role for vascular 20-HETE in the development of hypertension.

In addition to its actions on endothelial NO synthase, 20-HETE potently stimulates endothelial activation by activ-
ting the nuclear factor κB (NF-κB) and mitogen-activated
protein kinase-extracellular signal–regulated kinase 1/2 path-
ways, leading to increased levels of proinflammatory cyto-
kines and adhesion molecules.19 NF-κB is activated on phosphorylation of inhibitor of κB (IκB) by IκB kinase
(IKK), thus setting NF-κB free and marking the IκB proteins
for proteosomal degradation.28 A recent study by Cheng et
al20 demonstrated that 20-HETE–mediated endothelial NO synthase uncoupling is mediated through activation of IKK, suggesting the existence of a cross-talk between 20-HETE–
mediated endothelial dysfunction and activation. The present
study was undertaken to determine the temporal relationship
between vascular 20-HETE and NF-κB activation in the
androgen-treated rats and examined whether inhibition of
IKK, a key signaling step in 20-HETE-mediated endothelial
dysfunction and endothelial activation, attenuates androgen-
induced hypertension, a model of 20-HETE–dependent hyperten-

Materials and Methods
A detailed description of experimental protocols, methods, and
materials is included in the online Data Supplement (please see
http://hyper.ahajournals.org).

Results
Time Course of Increases in BP and Vascular
20-HETE in Dihydrotestosterone-Treated Rats
Administration of dihydrotestosterone (DHT) to normoten-
sive rats significantly increased systolic BP by day 3
(132.7±1.7 versus 117.2±0.8 mm Hg in DHT- and vehicle-
treated rats, respectively). By day 7, BP reached its maximal
levels (Figure S1A). The time course of the increase in renal
vascular levels of 20-HETE paralleled that of BP, demonstrat-
ing a 3-fold increase on day 3 of treatment (45.7±12.3
versus 12.6±1.8 ng/mg in DHT- and vehicle-treated rats,
respectively) and increasing thereafter to levels 4 times
greater than those measured before treatment (Figure S1B).
Additional experiments indicated that levels of 20-HETE
significantly increased as early as day 2 of DHT treatment
(57.7±9.7 versus 17.7±2.4 ng/mg at day 0), whereas a
significant increase in systolic BP was observed at day 3 of
treatment (Figure S2).

Inhibition of 20-HETE Synthesis or Action
Prevents and Attenuates Androgen-Dependent
20-HETE–Mediated Hypertension
To determine whether the hypertensive effect of androgen is
20-HETE dependent, the 20-HETE synthesis inhibitor,
HET0016, or the 20-HETE antagonist, 20-6,15-HEDE, was
either coadministered with DHT or administered to rats on
day 7 of DHT treatment. As seen in Figure 1A, cotreatment
of DHT with HET0016 prevented the BP increase to DHT.
Moreover, the DHT-induced increase in BP was greatly
attenuated by cotreatment with 20-6,15-HEDE; at day 10 of
cotreatment, BP decreased from 145.7±12.3 to 124.2±7.0
(Systolic BP [mm Hg])
20-6,15-HEDE after 7 days of DHT treatment resulted in a rapid decrease in BP. At day 2 of 20-6,15-HEDE administration, BP decreased from 141.9 ± 1.8 to 128.5 ± 2.2 mm Hg (Figure 1B) and remained significantly lower than that of the DHT-treated rats thereafter. In contrast, administration of HET0016 on day 7 of DHT treatment resulted in a gradual decrease in BP that was significantly different than that of the DHT-treated rats on day 15 of the experiment (Figure 1B).

Increased Renal Arterial Vascular Reactivity to DHT is 20-HETE Dependent
The increases in BP and vascular 20-HETE occurred within the first 3 days of DHT treatment. No differences in phenylephrine sensitivity were observed between arteries from vehicle- and DHT-treated rats at day 2 of treatment (data not shown). However, arteries from rats treated with DHT for 3 days displayed a significant increase in sensitivity to phenylephrine-induced vasoconstriction, as evidenced by a 3- to 5-fold increase in the EC_{50} to phenylephrine (Figure 2A). This increase in sensitivity was significantly attenuated in arteries from rats cotreated with 20-6,15-HEDE (Figure 2A).

Impaired Acetylcholine-Mediated Vasorelaxation to DHT is 20-HETE Dependent
Similar to vascular reactivity, endothelial dysfunction measured as impaired relaxing responses to acetylcholine was observed as early as 3 days of DHT treatment. As seen in Figure 2B, arteries from DHT-treated rats reached a maximum relaxation of 32.3 ± 5.7% versus 68.8 ± 5.4% in arteries from vehicle-treated rats. This decrease was attenuated in rats cotreated with 20-6,15-HEDE in vivo (57.6 ± 6.0% relaxation). Interestingly, a similar effect (53.0 ± 2.1% relaxation) was observed when 20-6,15-HEDE was added to the chamber of arteries from DHT-treated rats (Figure 2B).

DHT Treatment Leads to Endothelial Activation That Is 20-HETE Dependent
20-HETE has been shown to stimulate vascular endothelial NF-κB activation and cytokine production in vitro, effects that may contribute to the development of vascular inflammation that frequently accompanies endothelial dysfunction, as well as hypertension. Along with increasing vascular synthesis of 20-HETE, treatment with DHT also increased NF-κB activation. Thus, arteries from 3-day DHT treatment displayed a 3-fold increase in phosphorylated IκBα when compared with arteries treated with the vehicle (Figure 3A). Moreover, the increase in phosphorylated IκBα concurred with an increase in NF-κB transcriptional activity, because nuclear protein isolated from interlobar arteries of DHT showed a 50% increase in NF-κB (p65). Importantly, the increase in NF-κB (p65) was completely negated in arteries from rats treated with DHT and 20-6,15-HEDE (Figure 3B).
Inhibition of NF-κB Activation Attenuates Androgen-Dependent 20-HETE–Mediated Hypertension

Parthenolide has been used to selectively inhibit IKK activation. As seen in Figure 4A, parthenolide alone had no effect on BP; however, when administered together with DHT, it prevented the BP increase to DHT. At day 3 of the experiment, BP was 108.6±0.5, 109.8±1.7, 133.7±0.4, and 109.8±0.8 mm Hg in vehicle-, parthenolide-, DHT-, and DHT+parthenolide-treated rats, respectively. NF-κB activity in renal interlobar arteries showed a similar pattern; DHT treatment increased NF-κB activity by 45% when compared with vehicle treatment and cotreatment with parthenolide inhibited DHT-induced NF-κB activation (Figure 4B). Importantly, treatment with parthenolide did not affect the basal or the DHT-stimulated production of 20-HETE in the renal interlobar arteries (Figure 4C).

Parthenolide had a differential effect on vascular reactivity and relaxation. Whereas treatment with DHT increased vascular reactivity by 2.6- and 3.5-fold when compared with vehicle or parthenolide alone (Figure 5A), administration of parthenolide to DHT-treated rats did not significantly alter the vascular reactivity to phenylephrine (Figure 5A). In contrast, the relaxing response to acetylcholine, which was greatly diminished in arteries from rats treated with DHT alone, was fully restored in arteries from rats treated with DHT and parthenolide (from 23.4±7.4% to 50.9±1.3% relaxation at 5×10⁻⁶ m acetylcholine; Figure 5B). The relaxing response to sodium nitroprusside was not different among the groups, indicating that the vascular smooth muscle component of NO action is not altered by DHT and/or parthenolide (Figure 5C).

Discussion

This study demonstrates a close temporal relationship between increases in BP and vascular 20-HETE synthesis in the rat model of androgen-induced hypertension. Moreover, based on a recent study that identified IKK activation as the common signaling step in 20-HETE–mediated endothelial dysfunction and activation in vitro,20 this study is the first to demonstrate 20-HETE–mediated NF-κB activation in the
renal vasculature of androgen-treated rats and to show that administration of an IKK inhibitor prevents the androgen-mediated 20-HETE–dependent increase in BP.

Recent studies from our laboratory suggested that androgen-driven hypertension is mediated, in part, by increased vascular production of 20-HETE and that 20-HETE–mediated endothelial dysfunction contributes to the hypertension in this model.\textsuperscript{16} This conclusion was based on the demonstration that the 20-HETE synthesis inhibitor, HET0016, prevented the BP increase to androgen. However, a direct relationship between hypertension and the vascular actions of 20-HETE, including increased vascular reactivity, impaired endothelial-dependent relaxations, and activated endothelium, in the androgen model has not been established.

To this end, the use of the 20-HETE antagonist and the focus on the initial response to androgen in the present study provided a thorough examination of this relationship. Hence, the increase in BP in response to androgen paralleled the increase in renal vascular synthesis of 20-HETE; both increased at day 3 and were at their maximum increase at 5 to 7 days of treatment. However, a closer analysis revealed that the increase in 20-HETE appeared to precede that of BP, although this assumption needs further substantiation by radiotelemetry. Nevertheless, this suggested that 20-HETE may act as an initiating factor of androgen-driven hypertension.

Androgen has been identified as a potent inducer of CYP4A enzymes, including CYP4A2 and CYP4A8, which are the major 20-HETE–producing enzymes in the rat vasculature.\textsuperscript{29,30} It is possible that induction of CYP4A within the vasculature brings about an increase in 20-HETE levels, which, in turn, acts on the smooth muscle to increase vasoconstriction and on the endothelium to impair NO-dependent relaxation and to initiate the inflammatory program; all of these events, including the BP increase, appeared to follow the initial 2.5-fold increase in 20-HETE levels at day 2 of androgen treatment. This order of events, together with the ability of 20-HETE inhibitor or antagonist to prevent/reverse their occurrence, suggests that 20-HETE is a significant causative factor in this model. However, it does not rule out the possibility that the observed changes can occur as independent events influenced by multiple factors.

Androgen can modulate BP through multiple pathways. Along with increasing levels of 20-HETE, androgen can activate the renin-angiotensin-aldosterone system by increasing levels of angiotensinogen, hence modulating both kidney function and vascular function.\textsuperscript{5,31,32} In addition, androgen increases levels of phenylephrine, neuropeptide Y, and endothelin 1, as well as the expression of thromboxane A\textsubscript{2} receptors, all of which contribute to a pressor effect. These pressor mechanisms may be amplified by the presence of increased levels of 20-HETE. They may also explain the lack of a complete inhibition of the BP increase to DHT in the presence of a 20-HETE synthesis inhibitor or a 20-HETE activity blocker.

In a previous study, we showed that 20-HETE stimulates IκB phosphorylation and NF-κB translocation in endothelial cells.\textsuperscript{19} The demonstration that activation of NF-κB, in the form of increased IκB phosphorylation and NF-κB transcriptional activity, was observed as early as 3 days of DHT treatment and was negated by administration of the 20-HETE antagonist not only substantiated the in vitro data but also has pathophysiological implications. NF-κB is a proinflammatory transcription factor that plays an important role in vascular inflammation. Activation of NF-κB is a series of phosphorylation–dephosphorylation and cellular translocation processes that, in the end, turn on the transcription machinery of physiological and pathological mediators, including immune, acute phase, and proinflammatory genes.\textsuperscript{33} With the increasing evidence of a role for inflammation in the pathogenesis of hypertension,\textsuperscript{34} NF-κB–mediated signaling has emerged as a potential target for treatment of hypertension. Inhibitors of NF-κB activation, including pyrroolidinedithiocarbamate and parthenolide, have been shown to lower BP in several models of hypertension, including angiotensin II–treated rats, spontaneously hypertensive rats, and deoxycorticosterone-salt hypertensive rats.\textsuperscript{35–38} In these models, NF-κB inhibition was associated with an attenuation of renal interstitial inflammation and oxidative stress, as well as amelioration of renal injury.

Endothelial activation presents a proinflammatory phenotype in which the endothelium typically displays upregulation of cellular adhesion molecules, endothelial-leukocyte interaction, and alterations in the secretion of autocrine and/or paracrine factors, all of which contribute to inflammation. A recent study by Cheng et al\textsuperscript{20} suggested that 20-HETE–mediated endothelial activation and endothelial dysfunction are interdependent through IKK by demonstrating that inhibition of IKK activation in endothelial cells abrogated 20-HETE–mediated inhibition of NO. Our results indicate that vascular dysfunction and activation occur in the model of androgen-dependent hypertension; DHT treatment was associated with increased vascular reactivity, impaired relaxation to acetylcholine, and activation of NF-κB in response to DHT. That these were mediated by 20-HETE was evident from the ability of HET0016 or 20-6,15-HEDE to reverse and prevent the effect of DHT. Thus, endothelial dysfunction and activation seen in endothelial cells treated with 20-HETE in vitro seemed to occur in vivo, likely a result of increasing vascular levels of 20-HETE in response to androgen. Our study also indicates that IKK activation may be the link between these 2 processes in vivo. Administration of the IKK inhibitor parthenolide to DHT-treated rats not only reversed the increase in BP but also inhibited vascular NF-κB activation but also abrogated the increase in vascular reactivity and the impaired relaxation to acetylcholine. These results suggest that IKK activity may be the trigger that mediates both 20-HETE–mediated endothelial activation and dysfunction. However, because the parthenolide treatment is systemic, we cannot rule out the potential role of systemic NF-κB signaling and other IKK downstream signaling pathways, which may affect BP and vascular function. To this end, a study by Henke et al\textsuperscript{39} demonstrated that endothelial-specific NF-κB–suppressed mice placed on a high-salt diet and given angiotensin II together with N\textsuperscript{ω}-nitro-L-arginine methyl ester displayed reduced hypertension-induced renal damage despite high BP. Additional studies using vascular (endothelial)-specific suppression or deletion of IKK are important to better characterize the role of IKK in 20-HETE–mediated hypertension. Specific targeting is also important when considering the finding that, whereas partheno-
lide treatment prevented a DHT-mediated 20-HETE–dependent increase in BP and decrease in relaxation to acetylcholine, it did not affect the DHT-mediated increase in vascular reactivity to phenylephrine, which was fully negated by 20-6,15-HEDE. This suggested that the 20-HETE–dependent increase in vascular reactivity to phenylephrine is independent of IKK activation. This does not exclude the possibility that IKK is involved in a 20-HETE–mediated increase in sensitivity to other constrictor stimuli.

In summary, this study strongly supports the role of 20-HETE in mediating androgen-dependent hypertension and provides substantial evidence for its underlying prohypertensive vascular mechanisms. These mechanisms include IKK-dependent endothelial dysfunction and activation that are manifested by increased vascular reactivity and impaired endothelial-dependent relaxations that are sensitive to IKK inhibition.

Perspective

20-HETE has been identified as a primary microcirculatory eicosanoid of which the biological activities are conducive of a hypertensive factor. Numerous studies have documented a direct relationship between its vascular synthesis and BP in animal models of hypertension, and recent clinical studies suggested its involvement in human hypertension. Androgen has been implicated as a contributing factor to sex-specific differences in BP, and cardiovascular morbidity, and postmenopausal hypertensive activity of androgen. In this study, we showed that 20-HETE constitutes an initial step in the development of androgen-induced hypertension. Moreover, the androgen-mediated increase in vascular reactivity and endothelial dysfunction and activation was largely dependent on the initial increase in vascular 20-HETE’s synthesis. This study is also the first to implicate 20-HETE–mediated IKK activation, a step that links 20-HETE effects on the endothelial NO synthase-NO and cytochrome P450 expression and 20-HETE–mediated IKK activation, a step that links 20-HETE effects on the endothelial NO synthase-NO and cytochrome P450 expression and 20-HETE–mediated increase in sensitivity to other constrictor stimuli.

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Disclosures

None.

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Androgen-dependent hypertension is mediated by 20-HETE-induced vascular dysfunction: Role of IkappaB kinase

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Running Title: 20-HETE and IKK in androgen-dependent hypertension

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MATERIALS AND METHODS

Animal studies. All of the experimental protocols were performed following an Institutional Animal Care and Use Committee–approved protocol in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Sprague–Dawley male rats (8-9-week-old; Charles Rivers, Wilmington, MA) were administered DHT (56 mg/kg/day, ip) or its vehicle (20% benzyl alcohol in corn oil, ip) for up to 17 days. The indicated dose of DHT have been previously shown to induce hypertension in normotensive rats.1 In some experiments, rats were given the CYP4A selective inhibitor N-hydroxy-N’-(4-butyl-2-methylphenyl)-formamidine 2 (HET0016; 10 mg/kg/day in 10% w/v lecithin in saline, ip), or the 20-HETE antagonist, 20-6,15-HEDE 3 (10 mg/kg/day in 5% ethanol in saline, ip) either concomitantly or after 7 days of DHT treatment. In another set of experiments, rats were given the IKK inhibitor Parthenolide (1 mg/kg/day in DMSO, ip).4 For blood pressure measurements, rats were acclimated for seven days prior to the start of experiments. Systolic BP was determined by the tail cuff method (Kent Scientific). In brief, rats were placed on a far infrared heating pad for 7-10 minutes. Systolic blood pressure measurements were recorded after five cycles of acclimatization. At the end of experiments, rats were anesthetized with phenobarbital (50 mg/kg body wt, ip), and laparotomy was performed. The kidneys were removed, and renal interlobar arteries were microdissected for biochemical and functional studies.

Measurements of 20-HETE. Renal interlobar arteries were isolated from rats and incubated in oxygenated Krebs biocarbonate buffer, pH 7.4, with 1mM NADPH for 1 hour at 37°C with gentle shaking. 20-HETE was extracted and quantified by LC/MS/MS (Applied Biosystems, Foster City, CA) as previously described.5

Vascular function. Interlobar arteries (~230 µm, internal diameter) were cut into rings (~2 mm) and mounted on wires in the chambers of a multi-vessel myograph (JP Trading, Aarhus, Denmark) filled with Krebs’ buffer (37°C) gassed with 95% O2/5% CO2. After 30 to 60 min of equilibration, the vessels were set to an internal circumference equivalent to 90% of that which they would have in vitro when relaxed under a transmural pressure of 80 mm Hg. Isometric tension was monitored continuously before and after experimental interventions. A cumulative concentration-response curve to phenylephrine (10–9 to 5x10–5 mol/L) was constructed in the presence and absence of 20-6,15-HEDE (1 µmol/L). Phenylephrine-induced increase of isometric tension was expressed as the percentage of the increase in tension produced by the maximum contraction achieved. To determine acetylcholine-mediated vasorelaxation, vessels were rinsed with Krebs three times and incubated for 1 h followed by a final wash. After washing with Krebs buffer, vessels were preconstricted with phenylephrine and a cumulative concentration-response curve to acetylcholine (10–9 to 5x10–5 mol/L) was constructed in the presence and absence of 20-6,15-HEDE (1 µmol/L).

Western blot analysis. Western blot analysis of renal interlobar arterial segments were performed as previously described (27, 33 jenn AJP) using primary antibodies against phosphorylated IκBα (Cell Signaling, Beverly, MA), IκBα, VCAM (Santa Cruz Biotechnology, Santa Cruz, CA), and β-actin (Sigma, St. Louis, MO).

NF-κB activation. Nuclear and cytosolic proteins were extracted from microdissected renal interlobar arteries using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer’s instructions. Nuclear protein concentration was measured by the Bradford protein assay (Bio-Rad,
NF-κB activation was determined using the NF-κB p65 transcription factor assay (Cayman Chemical Company, USA.) according to the manufacturer's instructions.

Statistics. The data are presented as mean ± standard error (SE). Statistical significance (p<0.05) between the experimental groups was determined by the Fisher method of analysis for multiple comparisons. For comparison between treatment groups, the Null hypothesis was tested by a single factor analysis of variance (ANOVA; Dunnett's Multiple Comparison Test) for multiple groups or unpaired t-test for two groups.
Figure S1: Time course of increases in blood pressure and vascular 20-HETE in DHT-treated rats. (A) Systolic blood pressure (N=10-16). (B) 20-HETE levels in renal interlobar arteries (N=6-12). Results are mean±SE, *p<0.05 vs vehicle treated rats; #p<0.05 vs day 0; ‡p<0.05 vs DHT after 3 days; †p<0.05 vs DHT after 5 days.
Figure S2: Initial time course of blood pressure increase and vascular 20-HETE in DHT-treated rats. (A) Systolic blood pressure (N=6-12). (B) 20-HETE levels in renal interlobar arteries (N=6). Results are mean±SE; *p<0.05 vs day 0.
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