Role of NADPH Oxidase in the Vascular Hypertrophic and Oxidative Stress Response to Angiotensin II in Mice

Hui Di Wang, Shanqin Xu, Douglas G. Johns, Yue Du, Mark T. Quinn, Antonio J. Cayatte, Richard A. Cohen

Abstract—Oxygen-derived free radicals are involved in the vascular response to angiotensin II (Ang II), but the role of NADPH oxidase, its subunit proteins, and their vascular localization remain controversial. Our purpose was to address the role of NADPH oxidase in the blood pressure (BP), aortic hypertrophic, and oxidant responses to Ang II by taking advantage of knockout (KO) mice that are genetically deficient in gp91phox, an NADPH oxidase subunit protein. The baseline BP was significantly lower in KO mice than in wild-type (WT) (92±2 [KO] versus 101±1 [WT] mm Hg, P<0.01), but infusion of Ang II for 6 days caused similar increases in BP in the 2 strains (33±4 [KO] versus 38±2 [WT] mm Hg, P>0.4). Ang II increased aortic superoxide anion production 2-fold in the aorta of WT mice but did not do so in KO mice. Aortic medial area increased in WT (0.12±0.02 to 0.17±0.02 mm², P<0.05), but did not do so in KO mice (0.10±0.01 to 0.11±0.01 mm², P>0.05). Histochemistry and polymerase chain reaction demonstrated gp91phox localized in endothelium and adventitia of WT mice. Levels of reactive oxidant species as indicated by 3-nitrotyrosine immunoreactivity increased in these regions in WT but not in KO mouse aorta in response to Ang II. These results indicate an essential role in vivo of gp91phox and NADPH oxidase-derived superoxide anion in the regulation of basal BP and a pressure-independent vascular hypertrophic and oxidant stress response to Ang II. (Circ Res. 2001;88:947-953.)

Key Words: angiotensin II • superoxide anion • 3-nitrotyrosine • gp91phox • NADPH oxidase

Although angiotensin II (Ang II) mediates its effects on the vasculature directly after stimulation of AT1 receptors, recent publications suggest that an important part of its effects are by way of oxygen-derived free radicals, the production of which it stimulates.1,2 In addition, Zafari et al3 and Griendling et al4 have reported that NADPH oxidase mediates Ang II–induced hypertrophy of smooth muscle cells in culture.

Although a role of NADPH oxidase in Ang II–induced hypertension has been widely reported, the vascular location and the role of various subunit proteins of the oxidase have been controversial. NADPH oxidase subunits, including gp91phox, are expressed in endothelial cells in culture.5–7 Our group has shown that the adventitia is also an important site of superoxide anion production in normal rat6 and rabbit aorta,9 and NADPH oxidase subunits, including gp91phox, are found in native8 and cultured10 adventitial fibroblasts. The oxidase accounts for the majority of superoxide anion production in the adventitia, where it has been implicated in inactivating NO.8,9,11 Furthermore, superoxide anion is increased by Ang II–induced hypertension,1 particularly in the adventitia.9,11 In addition, we proposed that the increased superoxide anion generated in the aortic adventitia of Ang II–induced hypertensive rats was responsible for spontaneous myogenic tone, which was in part due to the inactivation of the endogenous vasodilator NO.12

NADPH oxidase has been studied most in leukocytes, where it is stimulated as part of the oxidative burst. Vascular NADPH oxidase differs in that it appears to be constitutively active, and its activity is stimulated by Ang II.13 A fibroblast NADPH oxidase was reported to have a cytochrome-containing subunit that differed from the gp91phox in the neutrophil NADPH oxidase.14 In addition, it has been reported that superoxide anion production by the aorta of the gp91phox mouse is the same as that of wild-type mice.15 Although a constitutively active homolog of gp91phox, MOX-1, has been cloned,16 and which might explain this discrepancy, its role in intact blood vessels is not yet known.

The purpose of this study was to further address the role of superoxide anion derived from NADPH oxidase in the pressor, vascular hypertrophic, and oxidant responses during Ang II–dependent hypertension. We took advantage of mice that are genetically deficient in gp91phox to determine the role of this specific subunit of the oxidase. These mice have been used previously to study the role of gp91phox and NADPH oxidase as an oxygen sensor in vivo.17 Our findings indicate

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that gp91phox is essential for Ang II–induced superoxide anion production, vascular hypertrophy, and oxidant stress.

Materials and Methods

Animal Model

Male gp91phox knockout and C57BL/6J control mice, 12 to 14 weeks of age were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) or inhaled isoflurane. An incision was made in the midscapular region under sterile conditions, and osmotic minipumps (Alzet model 1007D, Alza Corp) containing Ang II dissolved in 0.15 mol/L NaCl and 1 mmol/L acetic acid were implanted. The delivery rate was 3.2 mg/kg per day for 6 days. Sham-treated animals underwent an identical surgical procedure, except that an osmotic minipump containing 0.15 mol/L NaCl and 1 mmol/L acetic acid was implanted. Tetracycline (0.5 mg/mL) was given in the drinking water 24 hours before the surgery and continued until the end of the infusion. This antibiotic was given to the gp91phox-deficient animals because they are prone to infection, and it was given to the wild-type animals so as not to confound the comparison of the 2 groups. Systolic blood pressure was determined before and at the end of the drug infusion by tail-cuff plethysmography. Ten to 20 repeated values were averaged at each determination. The noninvasive method to measure blood pressure has been validated in mice and correlates well with intra-arterial measurements made in normotensive and hypertensive mice. These procedures were approved by the Boston University Medical Center Institutional Animal Care and Use Committee.

Detection of Superoxide Anion by Lucigenin Chemiluminescence

The details of this assay have been published previously. Briefly, after the aorta was isolated and cleaned of fat and loose connective tissue, the aorta was incubated in physiological buffer and maintained for 30 minutes at 37°C and pH 7.4 by gassing with 95% O2/5% CO2. The aorta was then transferred into test tubes containing 1 mL of HEPES-buffered physiological solution (pH 7.4) containing lucigenin (5 μmol/L). This lower concentration of lucigenin was used as compared to that of gp91phox-positive immunoreactivity for both 3-nitrotyrosine and gp91phox subunit. Specificity of anti–3-nitrotyrosine and gp91phox antibodies was confirmed by preincubation of antibody with free 3-nitrotyrosine (10 mmol/L) or by using a nonimmune rabbit IgG (Vector) isotypic control, respectively. Semi-quantitative analysis of tissue immunoreactivity for nitrotyrosine was done by 3 blinded observers using an arbitrary grading system from 1 to 4 to estimate the degree of positive staining.

Measurement of Aorta Medial Area

Two cross sections, each spaced 50 to 70 μm apart, were stained with hematoxylin and eosin and photographed at a magnification of ×100. The images from these microscopic sections were displayed on a computer using Photoshop software. The aortic media was then outlined on the image and measured using NIH Image software. The data from each of the 2 sections from each animal were averaged and expressed as the medial area per section. Four measurements of aortic medial thickness were also made by subtracting the internal and external diameters. These data reflected the same findings as the medial area and are not reported.

Real-Time Polymerase Chain Reaction (PCR) Analysis

Total RNA was isolated from endothelium-intact and endothelium-denuded wild-type mouse aorta using the SV total RNA isolation kit (Promega). Using the same technique, RNA was isolated from adventitial fibroblasts cultured as described. cDNA was synthesized using 200 units Superscript II RNase H–reverse transcriptase (Moloney murine leukemia virus, GIBCO-BRL) and 0.5 μg of total RNA primed with oligo-dT primer. After reverse transcription of RNA into cDNA, real-time PCR was performed with the SYBR Green I reporter system for the target sequences (gp91phox and endothelial NO synthase [eNOS]), and the TaqMan system for GAPDH using the ABI Prism 7700 Sequence Detection System according to the manufacturer’s instructions (Perkin-Elmer Applied Biosystems). Quantification and comparison of mRNA levels between endothelium-intact and endothelium-denuded aortic preparations were performed using the comparative C, method as described by the manufacturer.

Immunoblot Analysis of Mouse gp91phox

Mouse aortic adventitial fibroblasts were isolated and cultured (see expanded Materials and Methods section available online at http://www.circresaha.org). Immunoblotts for gp91phox protein in adventitial fibroblast lysates were performed by standard techniques on 20 μg of protein (see online data supplement). Reagents

Ang II, lucigenin, and Tiron were purchased from Sigma. Drugs were added in aliquots of <1% of the solution volume. All drugs were prepared freshly as stock solutions in distilled water.

Data Analysis

Data are expressed as mean±SEM. Statistical comparisons were made by 1- or 2-way ANOVA. Significance was accepted when P<0.05. An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

Baseline Body Weight and Blood Pressure

In 10- to 12-week-old mice, baseline systolic blood pressure was significantly lower in gp91phox knockout mice compared...
Ang II infusion was not significantly different in the gp91 phox knockout mice, the increase from baseline blood pressure to that after infusion in WT mice was not significantly different from that of knockout mice. 

Blood pressure obtained was significantly lower, independent of body weight, values in both groups (Table 2). After subtracting lucigenin with wild-type C57BL/6 mice (Table 1). The initial body weight was also slightly but significantly smaller in the knockout mice, each of which had a body weight of 26 g. The systolic blood pressure of mice in wild-type and gp91 phox knockout was related to the lower blood pressure, we compared the whether this slightly lower body weight of the knockout mice with wild-type C57BL/6 mice (Table 1). The initial body weight was also slightly but significantly smaller in the knockout mice, each of which had a body weight of 26 g. The systolic blood pressure of mice in wild-type and gp91 phox knockout was related to the lower blood pressure, we compared the whether this slightly lower body weight of the knockout mice

Table 1. Systolic Blood Pressure (mm Hg) in Experimental Mice

<table>
<thead>
<tr>
<th>Experimental Design</th>
<th>WT</th>
<th>gp91 phox Knockout</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT vs gp91 phox knockout</td>
<td>102 ± 2 (n=30)</td>
<td>92 ±2* (n=32)</td>
<td>0.01</td>
</tr>
<tr>
<td>Mice with body weight of 26 g</td>
<td>104 ± 2 (n=7)</td>
<td>89 ±3* (n=7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Before Ang II infusion</td>
<td>108 ±0.8 (n=11)</td>
<td>95 ±2* (n=10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After Ang II infusion</td>
<td>142 ±2 (n=11)</td>
<td>126 ±5* (n=10)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Change in pressure with Ang II infusion</td>
<td>38 ±2 (n=11)</td>
<td>33 ±4 (n=11)</td>
<td>&gt;0.4</td>
</tr>
</tbody>
</table>

Data are tail systolic pressure in wild-type (WT) and gp91 phox knockout mice. Blood pressure obtained was significantly lower, independent of body weight, before or after Ang II infusion. Change in pressure from before to after Ang II infusion in WT mice was not significantly different from that of knockout mice.

Pressor Responses to Ang II Infusion

Ang II infusion for 6 days increased systolic blood pressure in wild-type mice from 108.0 ± 0.8 to 142 ± 2 mm Hg, and in gp91 phox knockout mice from 95 ± 2 to 126 ± 5 mm Hg (Table 1). Whereas the blood pressure value reached after Ang II infusion was significantly lower in the gp91 phox knockout mice, the increase from baseline blood pressure to that after Ang II infusion was not significantly different in the gp91 phox and wild-type (Table 1) mice.

Superoxide Anion Levels in Mouse Aorta in Response to Ang II

Lucigenin chemiluminescence of aorta from Ang II–infused hypertensive C57BL/6J mice was significantly greater than that from sham-treated wild-type mice (Table 2). After adding Tiron, chemiluminescence was reduced to similar values in both groups (Table 2). After subtracting lucigenin chemiluminescence in the presence of Tiron from that obtained in its absence, the calculated Tiron-quenchable chemiluminescence was significantly higher in aorta from Ang II–infused mice and 2-fold greater than in the aorta of sham-treated normotensive mice (Table 2).

Chemiluminescence of aorta from sham-treated and Ang II–treated gp91 phox knockout mice was not significantly different (Table 2). After adding Tiron, the chemiluminescence was also not significantly different, such that the Tiron-quenchable chemiluminescence also was not significantly different in the 2 groups (Table 2). The Tiron-quenchable chemiluminescence of the aorta of sham-treated gp91 phox knockout mice was not significantly different from wild type (P>0.6), although there was a highly statistically significant difference in that of the aortas from Ang II–treated knockout and wild-type mice (P<0.002).

Localization of 3-Nitrotyrosine by Immunohistochemistry

Superoxide anion generated in the aorta can react with NO to form 3-nitrotyrosine protein moieties, which may be used as a marker of oxidative stress. In wild-type mice infused with Ang II, immunohistochemistry performed with a polyclonal antibody localized 3-nitrotyrosine staining to the adventitia and the endothelium (Figure 1). Lesser amounts of staining were observed in the media. Immunoreactivity was not observed if the anti–3-nitrotyrosine antibody was preincubated with 3-nitrotyrosine (10 mmol/L), indicating that the staining was specific. Semiquantitative analysis of the 3-nitrotyrosine staining performed by 3 blinded observers showed that staining in wild-type Ang II–infused mice was visibly increased compared with sham-treated mice (Figure 2). Staining of the aorta of sham-treated gp91 phox knockout mice was similar to that of wild-type mice, but no increase occurred in the aorta from Ang II–infused knockout mice.

Localization of gp91 phox by Immunohistochemistry and PCR

gp91 phox was evident primarily in aortic adventitia and endothelium, with much less staining in the media layer (Figure 3). The staining pattern did not differ appreciably between sham-treated and Ang II–infused mice. The pattern of staining for gp91 phox was similar to that for 3-nitrotyrosine.

Table 2. Superoxide Anion Production in Mouse Aortic Rings

<table>
<thead>
<tr>
<th></th>
<th>Wild Type</th>
<th>gp91 phox Knockout</th>
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<tr>
<td></td>
<td>Sham n=8</td>
<td>Ang II–Treated n=7</td>
</tr>
<tr>
<td>Lucigenin</td>
<td>7.2±0.9</td>
<td>12.0±1*†</td>
</tr>
<tr>
<td>Lucigenin+Tiron</td>
<td>3.3±1.0</td>
<td>2.9±0.7</td>
</tr>
<tr>
<td>Tiron-quenchable</td>
<td>4.1±0.7</td>
<td>9.4±2.1*†</td>
</tr>
</tbody>
</table>

Comparison of superoxide anion production in aorta of sham-treated and Ang II–infused wild-type and gp91 phox knockout mice. Values are chemiluminescence of the aorta in mU/mg×min⁻¹⁻¹. Infusion of Ang II for 6 days increased aortic superoxide anion production 2-fold in the aortas of wild-type mice but did not do so in gp91 phox knockout mice. Values are mean±SEM. Data represent responses of aortas from 7 to 8 mice.

*P<0.05 compared with sham-treated group.
†P<0.05 compared with Ang II–treated gp91 phox knockout mouse.
Staining for gp91<sup>phox</sup> was absent in the aorta of knockout mice, indicating the specificity of the antibody (Figure 3). To confirm that gp91<sup>phox</sup> was present in cells other than endothelial cells in the vascular wall of wild-type mice in situ, real-time quantitative PCR was performed. Although as expected, endothelial cell denudation significantly decreased eNOS mRNA in wild-type aorta, no significant decrease was observed in mRNA for gp91<sup>phox</sup>, which confirmed its presence in nonendothelial cells in the aortic wall (Figure 4). PCR also showed gp91<sup>phox</sup> mRNA was present in cultured mouse aortic fibroblasts (Figure 4).

To further verify that gp91<sup>phox</sup> is expressed in the adventitia, cell lysates obtained from cultured adventitial fibroblasts were subjected to SDS-PAGE and immunoblotting with polyclonal antibody directed against mouse gp91<sup>phox</sup>. Figure 4C shows an immunoreactive band that migrates at 77 kDa in mouse aortic adventitial fibroblast protein from wild-type but not gp91<sup>phox</sup> knockout mice.

### Hypertrophic Responses to Ang II Infusion

The medial cross-sectional area of sham-treated wild-type and gp91<sup>phox</sup> knockout mice was not significantly different (Figure 5, P>0.5). Ang II infusion significantly increased aortic medial area in wild-type mice (P<0.02), but no significant change occurred in gp91<sup>phox</sup> knockout mice infused with Ang II (Figure 5, P>0.4). Examples of the medial hypertrophy that occurred in response to Ang II in wild-type mouse aorta, and its absence in the aorta of knockout mice infused with Ang II, can be seen in Figures 1 and 3.

### Discussion

Our results indicate a requirement for the leukocyte-like gp91<sup>phox</sup> in the hypertrophic and oxidative stress response to Ang II. Although the existence of gp91<sup>phox</sup> in blood vessels has been questioned,<sup>13</sup> its expression has been well documented in endothelial cells in culture,<sup>6,7,24</sup> and Ang II was...
shown recently to increase gp91phox expression in the aorta of wild-type mice. In addition, the gp91phox knockout mouse aorta was demonstrated to have enhanced endothelium-dependent relaxation, suggesting that the subunit participates in superoxide anion generation that limits NO bioactivity in vascular cells in situ. In contrast to the latter finding, Souza et al found that untreated wild-type and gp91phox knockout mouse aorta in the presence of exogenous NADPH or NADH generates similar levels of superoxide anion. The present study confirmed this observation on basal superoxide anion production in single mouse aortas in the absence of added nicotinamide adenine nucleotides. The localization of gp91phox in the mouse aorta by immunohistochemistry was similar to that which we showed previously in the rat aorta, and the specificity of the antibody was confirmed in this study by absent staining in the aorta of knockout mice. Prominent staining was present in the endothelium and adventitia, but there was also staining in some smooth muscle cells. Using quantitative PCR, we showed that gp91phox is present in the sham-treated, wild-type mouse aorta even after removal of the endothelium. According to the immunohistochemical staining, this is primarily in adventitial fibroblasts, and we confirmed the presence of the NADPH oxidase subunit in cultured mouse aortic adventitial fibroblasts by PCR and by immunoblot. These results indicate that gp91phox is part of the NADPH oxidase that is present in native mouse vascular endothelium and adventitia, and probably in at least some smooth muscle cells. A homolog of gp91phox is expressed in cultured rat aortic smooth muscle, but it is unlikely that the PCR primers used to detect gp91phox in this study amplified MOX-1 mRNA, or that the antibody used cross-reacted with MOX-1, because of the fact that specific staining was absent in the knockout mouse aorta.

The essential functional role of gp91phox in the oxidant response to Ang II was indicated by our results showing that the increase in superoxide anion caused by Ang II was absent in the knockout mice. The fact that superoxide anion levels were not different in sham-treated knockout mice compared with wild-type mice indicates that other sources of superoxide anion are present under baseline conditions, but that the role of gp91phox and NADPH oxidase increases in response to Ang II.

We obtained further evidence that gp91phox is involved in the oxidative response to Ang II by evaluating nitrotyrosine staining in the aorta. Formation of nitrotyrosine provides evidence of the reaction of superoxide anion with NO to form the short-lived, potent oxidant peroxynitrite that can nitrosate tyrosine constituents of proteins. Nitrotyrosine was seen in sham-treated mouse aorta, as has been observed in other normal tissues, likely as a result of the fact that NO and superoxide anion are produced normally and react within the aortic wall under normal conditions, as we had previously suggested from physiological and pharmacological studies in the rat aorta. Sham-treated knockout animals had staining similar to that of sham-treated wild-type animals, further indicating that sources other than those containing gp91phox are responsible for superoxide anion generation under basal conditions. The pattern of the most intense staining in the endothelium and adventitia in both normal and Ang II–

**Figure 4.** gp91phox present in adventitial fibroblasts in mouse aorta RNA was isolated from endothelium intact or denuded wild-type mouse aorta, and real-time PCR was performed as described in Materials and Methods. A, Ethidium bromide–stained agarose gel depicting real-time PCR end products from mouse aorta RNA was isolated from endothelium intact or denuded preparations (n=4). Levels of gp91phox mRNA did not change when endothelium was removed. B (right), Comparison of eNOS mRNA levels between endothelium-intact and denuded preparations (n=4). Levels of eNOS mRNA were 6-fold lower in denuded aorta compared with intact preparations. C, Immunoblot analysis of mouse aortic adventitial fibroblasts subjected to SDS-PAGE and immunoblotting as described in Materials and Methods. The blot depicts a band that migrates at 77 kDa in wild-type mouse cell lysates but not in those from gp91phox knockout mouse cells. Blot represents 1 of 3 independent experiments with similar results.

**Figure 5.** Comparison of aortic medial area in aorta of sham-treated and Ang II–infused wild-type and gp91phox knockout mice. Aortic medial area increased in wild-type but not in gp91phox knockout mice infused with Ang II. Values are average area obtained in 2 cross sections from proximal descending mouse thoracic aorta (mean±SEM), *P<0.05 compared with sham-treated group. Each group contains data from 6 to 8 mice. Abbreviations as in Figure 1.
infused wild-type aorta was similar to that obtained in the rat and rabbit aorta2 using vital staining for superoxide anion with nitroblue tetrazolium. The fact that nitrotyrosine staining was not visibly increased in the knockout mouse aorta by Ang II also substantiates our finding that the gp91phox-containing NADPH oxidase is required for the increased formation of superoxide anion in response to Ang II in wild-type mice. Although the functional effects of tyrosine nitration are not addressed in this study, it has been shown that this chemical modification is likely to be important in the dysfunction of many vascular proteins such as superoxide dismutase27 and prostacyclin synthase.28

Ang II infusion causes an influx of leukocytes that are localized on the endothelial surface and in the adventitia,29 and deletion of a chemokine receptor was recently reported to prevent vascular hypertrophy in response to Ang II.30 Although leukocytes cannot account for the gp91phox or 3-nitrotyrosine present in the endothelium or adventitia of sham-treated mouse aorta, we cannot exclude the possibility that leukocyte gp91phox participates in the vascular response to Ang II. The observations that both cultured smooth muscle2 and adventitial fibroblasts10,25 respond to Ang II with increased expression of NADPH oxidase subunits and production of superoxide anion suggests that these cells do participate in the increased vascular superoxide anion and nitrotyrosine observed in response to Ang II.

Interestingly, knockout animals had lower baseline blood pressure compared with wild-type animals of the same age and weight. This observation suggests a role for gp91phox and superoxide anion in the maintenance of normal blood pressure. This could be a reflection of resting renin-angiotensin status in the mouse causing some low-level stimulation of the oxidase. What cell type(s) and location, in resistance arteries or elsewhere, could be responsible for this NADPH oxidase–dependent regulation of blood pressure is not known. One possibility is that NO, which may be partially inactivated in endothelial7 and adventitial cells8 by superoxide anion derived from the oxidase, mediates the lower pressure in the knockout mice. This interpretation remains speculative, because there was no decrease in basal superoxide anion production in the aorta in the knockout animals, but it is supported by the observation of improved NO bioactivity in aorta of the gp91phox knockout mouse.7 That the primary objective of this study was to determine the role of gp91phox in the oxidative stress response to Ang II in the aorta, which of course does not contribute significantly to blood pressure regulation. It should be noted that in a study of apolipoprotein E/p47phox knockout animals, the average blood pressure was 30% less than in mice with wild-type p47phox,31 although the results were not statistically significant, possibly because of the relatively small number of animals. Thus, the role of NADPH oxidase in blood pressure regulation will require the focus of future studies.

Despite the lower baseline blood pressure, the increase in blood pressure in response to Ang II was similar in wild-type and knockout mice, indicating that the pressor response to Ang II itself does not depend entirely on gp91phox or superoxide anion. This result in the mouse that lacks p67phox differs from that showing that treatment with superoxide dismutase did decrease the pressor response to Ang II in the rat.32

The failure of the knockout animals to develop medial hypertrophy does indicate an essential role of gp91phox and superoxide anion in Ang II–mediated medial hypertrophy in vivo. This could be due to superoxide anion itself; hydrogen peroxide; or reactants of these species, including peroxynitrite. Our observation that NADPH oxidase is required for the medial hypertrophic response to Ang II in vivo substantiates earlier studies in rat aortic smooth muscle cells in culture.3,4 Using subpressor doses of Ang II, other investigators33 showed that the pressor response to Ang II is not essential for medial hypertrophy. Our result showing that the hypertrophic response did not occur in knockout mice despite their pressor response to Ang II also suggests that the smooth muscle hypertrophic response occurs by pressure-independent mechanisms. Finally, the fact that NADPH oxidase is primarily localized in endothelial and adventitial cells supports the suggestion34 that oxidant stress in these cells exerts a paracrine influence over aortic smooth muscle.

Acknowledgments

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References


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