Kidney

Remodeling of Afferent Arterioles From Mice With Oxidative Stress Does Not Account for Increased Contractility but Does Limit Excessive Wall Stress

Lingli Li, Di Feng, Zaiming Luo, William J. Welch, Christopher S. Wilcox, En Yin Lai

Abstract—Because superoxide dismutase (SOD) knockout enhances arteriolar remodeling and contractility, we hypothesized that remodeling enhances contractility. In the isolated and perfused renal afferent arterioles from SOD wild type (+/+) and gene-deleted mice, contractility was assessed from reductions in luminal diameter with perfusion pressure from 40 to 80 mmHg (myogenic responses) or angiotensin II (10^{-6} mol/L), remodeling from media:lumen area ratio, superoxide (O_2^−) and hydrogen peroxide (H_2O_2) from fluorescence microscopy, and wall stress from wall tension/wall thickness. Compared with +/+ strains, arterioles from SOD1−/−, SOD2+/−, and SOD3−/− mice developed significantly (P<0.05) more O_2^− with perfusion pressure and angiotensin II and significantly increased myogenic responses (SOD1−/−: −20.7±2.2% versus −12.7±1.6%; SOD2+/−: −7.4±1.3% versus −12.6±1.4%; and SOD3−/−: −9.1±1.9% versus −15.8±2.2%) and angiotensin II contractions and ≥2-fold increased media:lumen ratios. Media:lumen ratios correlated with myogenic responses (r^2=0.23; P<0.01), angiotensin II contractions (r^2=0.57; P<0.0001), and active wall tension (r^2=0.19; P<0.01), but not with active wall stress (r^2=0.08; NS). Differences in myogenic responses among SOD3 mice were abolished by bath addition of SOD and were increased 3 days after inducing SOD3 knockout (−26.9±1.7% versus −20.1±0.7%; P<0.05), despite unchanged media:lumen ratios (2.01±0.09 versus 2.02±0.03; NS). We conclude that cytosolic, mitochondrial, or extracellular O_2^− enhance afferent arteriolar contractility and remodeling. Although remodeling does not enhance contractility, it does prevent the potentially damaging effects of increased wall stress. (Hypertension. 2015;66:550-556. DOI: 10.1161/HYPERTENSIONAHA.115.05631.) • Online Data Supplement

Key Words: hypertension ■ oxidative stress ■ reactive oxygen species ■ superoxide dismutase ■ vascular remodeling

Blood vessels in hypertensive humans1–3 or animal models4–5 characteristically have evidence of remodeling, endothelial dysfunction,2,6 and increased reactive oxygen species (ROS).7 Although these features predict subsequent cardiovascular disease,8–10 the effects of the remodeling on microvascular function are unclear.11,12 Folkow et al.11,13 proposed that repeated episodes of blood pressure elevation elicited vascular remodeling that enhanced vasoconstriction and sustained hypertension. Later studies identified a specific role for structural remodeling of the renal afferent arteriole in human and experimental hypertension.14–16 Thus, an autopsy study of kidneys from 1377 subjects reported that renal afferent arteriopathy, which entailed medial thickening and luminal narrowing, occurred in 97% of subjects known to be hypertensive, yet in <12% of those known to be normotensive, whereas remodeling was not prominent in arterioles from other sites.17 Moreover, Mulvany and colleagues18 reported that young spontaneously hypertensive rats whose renal afferent arterioles from nephrectomy specimens had a lesser diameter developed a higher blood pressure when adult. Indeed, a reduction in luminal diameter of renal afferent arterioles should increase renal vascular resistance and limit the glomerular filtration rate, thereby contributing to hypertension.

To gain further insight into the consequences of remodeling of renal afferent arterioles, arteriolar media:lumen area ratios from mice with gene deletions for superoxide dismutase (SOD) isoforms were related to the generation of ROS and to contractile responses to perfusion pressure (myogenic response) or to angiotensin II (Ang II). The SOD knock−/− mouse model provides prolonged increases in afferent arteriolar ROS, contractility, and remodeling without renal damage.7,19,20 Myogenic responses were assessed from reductions in luminal diameter of mouse isolated and perfused afferent arterioles during increases in perfusion pressure. Active wall tension (AWT) was calculated from the difference between wall tension developed in physiological solution and passive tension in Ca^{2+}-free solution with ethylene glycol-bis (2-aminoethylether)-N,N,N′,N′-tetraacetic. Myogenic and Ang II responses were

Received April 11, 2015; first decision April 26, 2015; revision accepted May 27, 2015.
From the Department of Medicine, Division of Nephrology and Hypertension, Hypertension, Kidney and Vascular Research Center, Georgetown University, Washington, DC (L.L., D.F., Z.L., W.J.W., C.S.W., E.Y.L.); and Department of Physiology, Zhejiang University School of Medicine, Hangzhou, China (E.Y.L.).
The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.115.05631/-/DC1.
Correspondence to En Yin Lai, Department of Physiology, Zhejiang University School of Medicine, Hangzhou 310058, China. E-mail laienyin@zju.edu.cn © 2015 American Heart Association, Inc.

Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.115.05631
related to remodeling and to the generation of arteriolar \( \mathrm{O}_2^- \), but these were dissociated by incubation of arterioles with pegalated-SOD or pegalated-catalase (to metabolize ROS without changing remodeling) or from short-term induction of SOD3 knockout (to increase ROS without remodeling), and AWS (wall thickness) normally increases with contractility and has been proposed to contribute to microvascular damage.\(^{21,22}\) Therefore, we related contractility to AWT and AWS to determine their roles in the functional adaption to a prolonged increase in afferent arteriolar ROS.

## Methods

### Animal Models

The first series used male littermate SOD1, SOD2, SOD3+/+, +/− and SOD1 and SOD3−/− mice (SOD2−/− is lethal). The second series used male mice 3 days after induction of SOD3 knockout with tamoxifen (3 mg/20g body weight, IP) compared with littersmates given vehicle.\(^{23}\) Mice were aged 3 to 4 months and weighed 25–30 g. All procedures conformed to the Guide for Care and Use of Laboratory Animals prepared by The Institute for Laboratory Animal Research. Studies were approved by the Georgetown University Animal Care and Use Committee.

### Isolation and Perfusion of Mouse Afferent Arterioles and Measurements of Superoxide and Hydrogen Peroxide

As described previously\(^{24,25}\) and in Methods in the online-only Data Supplement, superoxide (\( \mathrm{O}_2^- \)) generation was assessed by fluorescence microscopy from pegalated-SOD inhibitable ethidium/dihydroethidium fluorescence ratio (\( \mathrm{f/f}_0 \)) and hydrogen peroxide (\( \mathrm{H}_2\mathrm{O}_2 \)) from pegalated-catalase inhibitable 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (\( \mathrm{C}-\mathrm{H}_2\mathrm{DCFDA} \)) fluorescence, as described.\(^{26}\)

### Renal SOD Protein Expression

This was assessed by Western analysis of lysates of renal cortex as described\(^{27}\) (see Methods in the online-only Data Supplement).

### Determination of Passive Wall Compliance

This was assessed from passive wall stress/strain relationships as described\(^{28}\) (see Methods in the online-only Data Supplement).

### Statistics

Data were obtained from 5 to 7 mice per group and analyzed by ANOVA (with repeated measures for Ang II) to assess individual effects of genotype, intervention (perfusion pressure or Ang II), and interaction (effects of genotype on the response to the intervention). Where appropriate, a post hoc Bonferroni test was used to test differences between groups. Results (mean±SEM values) are considered significant at \( P<0.05 \).

## Results

The SOD protein expression was studied in renal cortical lysates to determine whether genetic deletion of 1 SOD isoform caused adaptive changes in renal expression in other SOD isoforms. Compared with littermate +/+ mice, the expression of the corresponding SOD isoforms was almost absent in SOD1 or SOD3−/− mice and was approximately one half in +/− mice (Figures S1 and S2 in the online-only Data Supplement). There were no apparent compensations by 1 SOD isoform for the loss of another.

Pegalated-SOD (200 \( \mathrm{U/mL} \)) blocked >80% of the ethidium/dihydroethidium fluorescence and pegalated-catalase (1000 \( \mathrm{U/mL} \)) blocked >80% of the \( \mathrm{C}-\mathrm{H}_2\mathrm{DCFDA} \) fluorescence in SOD3 mice (Figure S3A and S3B). Compared with arterioles from littermate +/+ mice, the increases in afferent arteriolar \( \mathrm{O}_2^- \) with perfusion pressure from 40 to 80 mmHg or with \( 10^{-6} \) mol/L Ang II were enhanced significantly in arterioles from SOD1−/−, SOD2−/−, or SOD3−/− mice (Figure 1A and 1B). The increases in \( \mathrm{O}_2^- \) in arterioles from SOD2+/− mice were generally similar to those in SOD1−/− and SOD3−/−, but there were no differences between SOD1 or SOD3+/+ and +/− mice. An increase in perfusion pressure did not increase \( \mathrm{H}_2\mathrm{O}_2 \) significantly in afferent arterioles from SOD3+/+ mice but did increase it in SOD3−/− mice (Figure 1C). A strictly similar increase in \( \mathrm{H}_2\mathrm{O}_2 \) with perfusion pressure was also seen in arterioles from SOD1−/− mice compared with SOD3−/− mice (7.8±1.5% versus 7.4±0.5%). Compared with arterioles from littermate +/+ mice, contractility was increased significantly in response to perfusion pressure (Figure 1D) or Ang II (Figure 1E) in arterioles from SOD1−/−, SOD3−/−, and SOD2+/− mice. Bath addition of pegalated-SOD prevented the enhanced contractions of SOD3−/− mouse arterioles to perfusion pressure (Figure 2A; without changing remodeling), whereas pegalated-catalase was ineffective (Figure 2B). Arteriolar \( \mathrm{O}_2^- \) generation was correlated with contractions to both perfusion pressure (\( r^2 =0.39; \ P<0.05 \)) and Ang II (\( r^2 =0.85; \ P<0.0001 \); Figure S4A and S4B). This analysis used group mean data because ROS and myogenic responses could not be measured in the same arterioles.

We had shown previously that blockade of NO synthase (NOS) with \( \mathrm{N}^{\text{(o)}}-\)nitro-l-arginine methyl ester (L-NAME) increased the sensitivity and responsiveness of afferent arterioles to Ang II in SOD1+/+ but not in SOD1−/− mice and abolished differences between genotypes.\(^ {7,19} \) Thus, Ang II responses were not repeated. Myogenic contractility also was enhanced by NOS inhibition in SOD1+/+ but an enhanced myogenic contractility in vessels from SOD1−/− mice persisted during L-NAME (Figure S5).

Compared with arterioles from littermate +/+ mice, those from SOD2+/− and SOD1−/− and SOD3−/− mice had an increased media wall area and a reduced vessel lumen area that resulted in striking and uniform increases by >2-fold in media:lumen ratios (Figure 3A–3C). These structural changes persisted in \( \mathrm{Ca}^{2+} \)-free solution which abolished vascular tone. The basal arteriolar lumen diameters are shown in Table S1.

AWT (dynes/cm) increased lineally with perfusion pressure as in prior studies.\(^ {24,25} \) Compared with littermate +/+ mice, the slope of the regression of AWT on perfusion pressure (to measure myogenic responses) was increased significantly in arterioles from SOD1−/−, SOD2+/−, and SOD3−/− mice (SOD1: 3.49±0.30 versus 2.30±0.27; \( P<0.05 \); SOD2: 3.00±0.19 versus 1.77±0.22; \( P<0.05 \); SOD3: 3.51±0.20 versus 2.43±0.15; \( P<0.01 \); Figure 4A; Table S2). AWS (dynes/cm) also increased with perfusion pressure but, unlike AWT, there were no differences between littermate and SOD gene-deleted mice (Figure 4B; Table S3).

After pooling individual data points across genotypes, changes in arteriolar diameter with perfusion pressure or Ang II were positively correlated with afferent arteriolar media:lumen ratio (perfusion pressure, \( r^2 =0.23; \ P<0.001; \)
Hypertension September 2015

Figure 5A and Ang II, \( r^2 = 0.57; P < 0.0001 \); Figure 5D). During increased perfusion pressure, AWT depended significantly on media:lumen ratio \( (r^2 = 0.19; P < 0.01; \text{Figure 5B}) \), whereas AWS was independent \( (r^2 = 0.08; \text{NS}; \text{Figure 5C}) \).

Compared with arterioles from littermate mice with the same genotype given vehicle, the afferent arterioles 3 days after induction of SOD3 knockout with tamoxifen were not remodeled (Figure 6A) but developed a similarly enhanced generation of \( \text{O}_2^- \) with perfusion pressure or Ang II (Figure 6B) and similarly increased contractions with perfusion pressure (Figure 6C) or Ang II (Figure 6D) as arterioles from lifetime SOD3−/− mice.

The passive arteriolar wall compliance, which was assessed from passive wall stress/strain relationships, was unaffected by SOD genotype (Figure S6).

**Discussion**

These studies confirm that SOD1−/− mice have afferent arteriolar remodeling and increases in \( \text{O}_2^- \) generation and contractile responses to Ang II. The main new findings are that there were no compensatory upregulations in renal expression of other SOD isoforms in SOD1, SOD2, or SOD3 gene-deleted mice. There were similar increases in afferent arteriolar remodeling, \( \text{O}_2^- \) generation, and contractile responses to perfusion pressure and Ang II among afferent arterioles from SOD1−/−, SOD2+/−, and SOD3−/− mice. The sharp increase in \( \text{O}_2^- \) with perfusion pressure in arterioles from SOD3−/− deleted mice accounted for their enhanced contractility because metabolism of \( \text{O}_2^- \) by pegalated-SOD normalized the myogenic responses (without a change in media:lumen ratio), whereas the increase in \( \text{H}_2\text{O}_2 \)

![Figure 1. Deletion of superoxide dismutase (SOD) genes enhances afferent arteriolar generation of reactive oxygen species and contractility. Changes in superoxide with perfusion pressure (A), angiotensin II (Ang II; B), hydrogen peroxide with perfusion pressure (C), contraction with perfusion pressure (D), or Ang II (E) in arterioles from SOD1 (open boxes), SOD2 (cross-hatched boxes), or SOD3 (solid boxes) gene manipulated mice (mean±SEM; n=5–7 per group). Compared with corresponding +/+ mouse vessels; *P<0.05; **P<0.01; and ***P<0.005. E:DHE indicates ethidium:dihydroethidium; H2DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; MR, myogenic response; and PP, perfusion pressure.](http://hyper.ahajournals.org/)

![Figure 2. A and B, Changes in diameter of afferent arterioles with perfusion pressure in the presence of pegalated agents. Comparing vessels from superoxide dismutase 3 (SOD3) +/- (open symbols) with SOD3−/− (solid symbols) mice incubated with vehicle (square symbols) or pegalated agents (circular symbols). Compared with SOD3+/+, †††P<0.005. MR indicates myogenic response; and PEG, pegalated.](http://hyper.ahajournals.org/)
was not sufficient to affect myogenic responses significantly because they were unaffected by metabolizing H₂O₂ with pegalated-catalase. The myogenic responses of arterioles from SOD3−/− mice remained stronger than SOD3+/+ mice during NOS blockade. Myogenic responses increased with O₂− but AWS was maintained because increases in tension in gene-deleted mice were balanced by equivalent increases in wall thickness.

Afferent arterioles from mice 3 days after inducing SOD3 knockout had similar increases in O₂− and contractions with perfusion pressure and Ang II as those from lifelong SOD3−/− mice but lacked any change in arteriolar media:lumen ratio. This, and the finding of similar myogenic responses in SOD3+/+ and−/− mice during metabolism of O₂− despite a maintained enhanced media:lumen ratio in the knockout mice, dissociates the increases in contractile responses in SOD3−/− mice from vessel remodeling. Finally, passive vessel wall compliance was preserved in SOD1−/−, SOD2+/−, and SOD3−/− mice. Thus, both short- and long-term gene deletions of SODs increase afferent arteriolar O₂− generation which is implicated in an increased contractility to perfusion pressure or Ang II. The remodeling of the vessel wall that develops in the long-term does not account for the increased contractility but does permit an increase in contractility without an increase in AWS. An increase in vascular wall stress is a stimulus for cell growth and can be provoked by an increase in myogenic tone.31 However, the medial hypertrophy does not seem to cause the enhanced myogenic tone and apparently does not make a dominant contribution to hypertension because SOD1, SOD2, and SOD3 gene-deleted mice all had prominent vascular remodeling, yet have generally been reported to be normotensive.19

Figure 3. A to C, Deletion of superoxide dismutase (SOD) genes enhances afferent arteriolar remodeling in SOD1 (open boxes), SOD2 (cross hatched boxes), or SOD3 (solid boxes) gene manipulated mice. Compared with corresponding +/+ mouse vessels: *P<0.05; **P<0.01; and ***P<0.005.

Figure 4. A and B, Active wall tension (AWT) and active wall stress (AWS) increase with perfusion pressure in superoxide dismutase (SOD)+/+ (solid circles and continuous lines), +/- (open triangles and dashed lines), or −/− (open squares and dotted lines) mice. P values refer to ANOVA with repeated measures for differences between genotypes.
A strong afferent arteriolar contraction is a key component of renal autoregulation, which maintains a constant renal blood flow, glomerular filtration rate, and glomerular capillary pressure during changes in blood pressure. These adjustments of preglomerular vascular tone prevent transmission of the hypertension into the glomeruli, thereby damaging podocytes and the renal parenchyma. The SOD3−/− mouse has increased excretion of 8-isoprostane F2α and increased renal vascular resistance, consistent with the increased O2·− and tone in isolated afferent arterioles in this study. Ang II generates both arteriolar NO that blunts the contraction and O2·− that bioinactivates NO. The enhanced Ang II contractions in afferent arterioles from SOD1−/− mice were ascribed to the enhanced NO bioinactivation because they were normalized during NOS inhibition. In contrast, a stronger myogenic response persisted in arterioles from SOD1−/− mice during NOS blockade. Thus, all of the enhanced Ang II contractility, but only about one half of the enhanced myogenic responses, in arterioles from SOD1−/− mice can be ascribed to NO bioinactivation. This is consistent with strong modulation of afferent arteriolar contractions to Ang II, thromboxane, or endothelin-1 by NO but a minor role of endothelial NOS in modulation of myogenic responses and no effect of endothelial NOS deletion.

Although arterioles from SOD3−/− mice generated more H2O2 with perfusion pressure, the enhanced contractility likely relates primarily to the enhanced generation of O2·− because deletion of p47phox, which is a prominent source of O2·−, prevents myogenic responses and pegalated-SOD, but not pegalated-catalase, prevented the exaggerated myogenic responses in arterioles from SOD3−/− mice. The enhanced generation of H2O2 in arterioles from SOD3−/− mice may reflect metabolism of increased O2·− by other SOD isoforms. However, aortic vascular remodeling is reduced in NOX2 gene-deleted mice and is prevented in vascular smooth muscle cell catalase transgenic mice. Thus, enhanced vascular contractility during oxidative stress is dependent on O2·−, whereas remodeling can be dependent on H2O2, thereby further dissociating contractility from remodeling.

In conclusion, afferent arterioles from SOD gene-deleted mice generate more O2·− with increased perfusion pressure and Ang II, which accounts for enhanced contractions. Their vessels are prominently remodeled but the enhanced contractions can be ascribed to O2·−, whereas the remodeling permits an
enhanced AWT during increased perfusion pressure without the potentially damaging effects of increased wall stress.

**Perspectives**

Results from this study do not support the hypothesis that remodeling of resistance vessels sustains hypertension.11,13 Indeed, careful studies in SOD gene-deleted mice generally report consistent remodeling,5,7 yet normal basal levels of blood pressure,19 although the early increase in blood pressure with Ang II is usually enhanced, consistent with the increased Ang II contractions of their afferent arterioles in this study.7,19 Sustained hypertension requires renal Na+ and fluid retention to prevent the corrective effects of a pressure natriuresis.45,46 Whereas ROS enhance Na+ reabsorption in the loop of Henle,47 sustained hypertension requires renal Na+ and fluid retention report consistent remodeling,7 yet normal basal levels of blood pressure,19 although the early increase in blood pressure with Ang II is usually enhanced, consistent with the increased Ang II contractions of their afferent arterioles in this study.7,19 Sustained hypertension requires renal Na+ and fluid retention to prevent the corrective effects of a pressure natriuresis.45,46

**Acknowledgments**

We thank David Harrison, MD (University of Vanderbilt, TN) for a generous gift of inducible SOD3−/− mice and Kathryn Windels for preparing the article.

**Sources of Funding**

This work was supported by research grants to E.Y. Lai from the National Nature Science Foundation of China (31471100) and Zhejiang Province Natural Science Foundation (LY13H070002) and to C.S. Wilcox from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; DK-49870 and DK-36079) and the National Heart, Lung, and Blood Institute (HL-68686) and from the George E. Schreiner Chair of Nephrology, the Smith/Kogod Family Foundation, and the Georgetown University Hypertension, Kidney and Vascular Research Center. Z. Luo was supported by a National Institutes of Health training grant (DK-59274).

**Disclosures**

None.

**References**


Remodeling of Afferent Arterioles From Mice With Oxidative Stress Does Not Account for Increased Contractility but Does Limit Excessive Wall Stress
Lingli Li, Di Feng, Zaiming Luo, William J. Welch, Christopher S. Wilcox and En Yin Lai

_Hypertension_. 2015;66:550-556; originally published online June 22, 2015;
doi: 10.1161/HYPERTENSIONAHA.115.05631
_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/66/3/550

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2015/06/22/HYPERTENSIONAHA.115.05631.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/
SUPPLEMENTAL METHODS

Remodeling of afferent arterioles from mice with oxidative stress does not account for increased contractility but does limit excessive wall stress

Lingli Li, MD, Ph.D1, Di Feng, Ph.D1, Zaiming Luo, MD1, William J. Welch, Ph.D1,
Christopher S. Wilcox, MD, Ph.D1 and En Yin Lai, MD, Ph.D1,2

1. Hypertension, Kidney and Vascular Research Center and Division of Nephrology and Hypertension, Department of Medicine, Georgetown University, Washington, DC 20007, USA
2. Department of Physiology, Zhejiang University School of Medicine, Hangzhou 310058, China

Address for correspondence:

En Yin Lai, MD, Ph.D.
Department of Physiology, Zhejiang University School of Medicine, Hangzhou 310058, China
Email: laienyin@zju.edu.cn
Phone: 86-13646877459
Fax: 1-877-625-1483

June 19, 2015
Supplemental Methods: Isolation and perfusion of mouse afferent arterioles and measurements of $O_2^-$ and $H_2O_2$: After euthanasia, single afferent arterioles with attached glomerulae were dissected from the kidney. The arteriolar lumen was perfused via a pipette containing an internal pressure pipette and the glomerulus stabilized by a suction pipette in an organ bath at 37°C on the stage of an inverted microscope, as described in detail 1-4.

Measurement of arteriolar contractility: The PP was increased in 20 mmHg steps from 40 to 140 mmHg. The arteriolar diameter was measured directly to assess MRs. Other mouse arterioles were prepared similarly and perfused at 60 mmHg during bath addition of graded concentrations of ANG II (10^{-12} - 10^{-6} mol\cdot l^{-1}). The luminal diameters were measured in real time after 2 minutes at each PP or dose of ANG II 5. To assess the contribution of nitric oxide (NO), N^0-Nitro-L-arginine methyl ester (L-NAME; 10^{-4} mol\cdot l^{-1}) or vehicle were added to the bath and the MRs assessed after 5 minutes. We have established that L-NAME increases the ANGII sensitivity and responsiveness of afferent arterioles from SOD1+/+ but not SOD1 -/- mice and abolishes differences between genotypes 6 Therefore, effects of LNAME were confined to MRs.

Assessment of arteriolar remodeling: The experiments were recorded by a video system, digitized, and monitored in real time to measure the arteriolar luminal diameter (d) and outer diameter (D) at 60 mmHg to approximate to physiological PP. Wall thickness (t) was calculated as \( t = D - d \); lumen area (L) was calculated as \( L = \pi (d/2)^2 \); media area (M) was calculated as \( M = \pi (D/2)^2 - \pi (d/2)^2 \). Remodeling was determined from the M:L ratio 4.

Determination of active wall tension and stress: Wall tension (T) was calculated from: \( T = P_i \times R \), where \( P_i \) is the intravascular perfusion pressure and R is the internal radius. \( T \) was first measured in physiological solution and thereafter in a Ca^{2+}-free bath solution containing 5 \times 10^{-3} mol\cdot L^{-1} ethylene glycol-bis (2-aminoethylether)-N,N',N'-tetraacetic acid (EGTA, Sigma, St. Louis, MO) to abolish active tone. The active wall tension (AWT) was calculated as the difference between the wall tensions measured during physiological solution (T_{physiol}) and passive tension in Ca^{2+}-free condition (T_{passive}) 1. The active wall stress (AWS) was calculated as AWT per unit wall thickness (AWS = AWT/t) 6.

Determination of passive wall compliance: This was assessed from passive wall stress/strain relationships. Afferent arterioles were perfused in Ca^{2+}-free solution with EGTA. The luminal and medial diameters were measured during graded PP. Passive wall stress was calculated as passive wall tension per unit wall thickness (PWS = T_{passive}/t). Passive wall strain was calculated from the ratio of change in luminal diameter to the diameter at maximum PP (Strain = \Delta D/D) 7.

Western analysis of SOD isoform expression in kidney cortex: Western blot was performed as previous described 8. Briefly, kidney cortical tissue was homogenized and protein concentrations were determined. Samples were loaded onto a 10%-20% Tris SDS-PAGE gel (BIO-RAD) and transferred to a PVDF membrane (BIO-RAD). After blocking with 5% nonfat dry milk, membranes were incubated with a primary antibody at room temperature for 2 hour, and then incubated with a secondary antibody at room temperature for 1.5 hour. The membranes were developed with ImmunStarTM HRP peroxide reagent (BIO-RAD). Signals were detected by FluorChem E (protein simple). The intensities of the target bands were quantified using Image J program. \( \beta \)-actin (Santa Cruz) was used for a loading
control. Primary antibodies were: SOD1 (Santa Cruz Biotechnology), 1:5000; SOD2 (Abcam), 1:5000; SOD3 (R&D Systems), 1:1000.
References


Table S1. Basal luminal diameter of afferent arterioles perfused at 60 mmHg

<table>
<thead>
<tr>
<th>Diameter (µm)</th>
<th>SOD genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>SOD1</td>
<td>9.46 ± 0.70</td>
</tr>
<tr>
<td>SOD2</td>
<td>11.39 ± 0.76</td>
</tr>
<tr>
<td>SOD3</td>
<td>9.61 ± 0.57</td>
</tr>
</tbody>
</table>

Compared to corresponding +/+: *, P<0.005; †, P<0.05.

Table S2. Slopes of the regression of active wall tension on perfusion pressure

<table>
<thead>
<tr>
<th>Slope (dynes.cm(^{-1}).mmHg(^{-1}))</th>
<th>SOD genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>SOD1</td>
<td>2.30 ± 0.27</td>
</tr>
<tr>
<td>SOD2</td>
<td>1.77 ± 0.22</td>
</tr>
<tr>
<td>SOD3</td>
<td>2.43 ± 0.15</td>
</tr>
</tbody>
</table>

Compared to corresponding +/+: *, P<0.05; †, P<0.01.

Table S3. Active wall stress of afferent arterioles perfused at 140 mmHg

<table>
<thead>
<tr>
<th>AWS (x10(^{-6}) dynes.cm(^{-2}))</th>
<th>SOD genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>SOD1</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td>SOD2</td>
<td>0.78 ± 0.11</td>
</tr>
<tr>
<td>SOD3</td>
<td>0.80 ± 0.11</td>
</tr>
</tbody>
</table>
Figure S1: Protein expression for SOD isoforms in renal cortical lysates from SOD +/+ (open boxes), +/− (cross-hatched boxes) or −/− (solid boxes) gene manipulated mice. Compared to corresponding +/+ mouse kidneys: *, P<0.05; ***, P<0.005. Compared to corresponding +/− mouse kidneys: †, P<0.05, ††, P<0.01.
Figure S2: Individual Western blots from renal cortical lysates.
Figure S3: Changes in fluorescence for ethidium: dihydroethidium (E:DHE) (Panel A) or 6-carboxy-2’,7’-dichlorodihydrofluorescein diacetate (H2DCFDA) (Panel B) in afferent arterioles from SOD3+/+ (open boxes) or SOD3-/- (solid boxes) mice during increases in perfusion pressure (PP; from 40 to 80 mmHg) or angiotensin II (ANG II; 10^-6 mol·l^-1) with vehicle or pegalated superoxide dismutase (PEG-SOD; 200 units·ml^-1) or PEG-catalase (1000 units·ml^-1).

Compared to baseline (PP=40 mmHg), *, P<0.05. Compared to SOD3+/+, †, P<0.05.
Figure S4: Correlation of group mean values for changes in diameter with superoxide generation during increases in perfusion pressure from 40 to 80 mmHg (Panel A) or 10^-6 mol·l^-1 angiotensin II (Panel B) in arterioles from SOD1 (circles), SOD2 (triangles), stable SOD3 (squares) or inducible SOD3 (diamond), +/- (solid), +/- (half shaded) or -- (open) gene deleted mice.
Figure S5: Changes in diameter with perfusion pressure in SOD1 gene manipulated mice for afferent arterioles incubated with vehicle (solid symbols) or $10^7$ mol·l$^{-1}$ L-NAME (open symbols) from SOD1 $+$/+ (circles) or SOD1 $-$/- (squares) mice. Comparing groups: *, P<0.05; ***, P<0.005.
Figure S6: Passive vessel walls stress/strain relationship in +/+ (solid symbols) or -/- (+/- for SOD-2) (open symbol) gene manipulated mice. P values refer to ANOVA for repeated measures comparing genotypes.