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Increased Hydrogen Peroxide Impairs Angiotensin II Contractions of Afferent Arterioles in Mice after Renal Ischemia-Reperfusion Injury

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Abstract

Aim—Renal ischemia reperfusion injury (IRI) increases angiotensin II (Ang II) and reactive oxygen species (ROS) that are potent modulators of vascular function. However, the roles of individual ROS and their interaction with Ang II are not clear. Here we tested the hypothesis that IRI modulates renal afferent arteriolar responses to Ang II via increasing superoxide ($O_2^{\cdot-}$) or hydrogen peroxide (H_2O_2).

Methods—Renal afferent arterioles were isolated and perfused from C57BL/6 mice 24 hours after IRI or sham surgery. Responses to Ang II or norepinephrine were assessed by measuring arteriolar diameter. Production of H_2O_2 and $O_2^{\cdot-}$ were assessed in afferent arterioles and renal cortex. Activity of SOD and catalase, and mRNA expressions of Ang II receptors were assessed in pre-glomerular arterioles and renal cortex.

Results—Afferent arterioles from mice after IRI had a reduced maximal contraction to Ang II ($-27 \pm 2\%$ versus $-42 \pm 1\%$, $P < 0.001$), but retained a normal contraction to norepinephrine. Arterioles after IRI had a 38% increase in H_2O_2 ($P < 0.001$) and a 45% decrease in catalase activity ($P < 0.01$). Contractions were reduced in normal arterioles after incubation with H_2O_2 ($-22 \pm 2\%$ versus $-42 \pm 1\%$, $P < 0.05$) similar to the effects of IRI. However, the impaired contractions were normalized by incubation with PEG-catalase despite a reduced AT_1R expression.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests in relation to the publication of this paper.

Conclusions—Renal IRI in mice selectively impairs afferent arteriolar responses to Ang II because of H_2O_2 accumulation that is caused by a reduced catalase activity. This could serve to buffer the effect of Ang II after IRI, and may be a protective mechanism.

Keywords

angiotensin II; hydrogen peroxide; catalase; afferent arteriole; ischemia reperfusion injury

INTRODUCTION

Ischemia reperfusion injury (IRI) is a largely unpreventable condition characterized by a decreased glomerular filtration rate (GFR) and renal blood flow (RBF), renal parenchymal damage and ongoing renal injury. IRI is a common cause of acute kidney injury (AKI) that contributes not only to mortality in the short term but also to the development of chronic kidney disease (CKD), end stage renal disease (ESRD) (Chawla et al., 2011), and cardiovascular disease (CVD) in the long term. Vascular dysfunction contributes to renal IRI (Gourdin et al., 2009). In particular, the reduced RBF and GFR that can be observed during the recovery phase are associated with changed renal arteriolar function. IRI is known to be accompanied by increased reactive oxygen species (ROS) that is a cause of the tissue injury seen after ischemia reperfusion (Brodsky et al., 2002, Sun et al., 2004). However, ROS influence renal vascular function in various ways (Carlstrom et al., 2010, Lai et al., 2012, Carlstrom et al., 2015, Li et al., 2015). NADPH oxidase derived superoxide ($\text{O}_2^{\cdot-}$) mediates angiotensin II (Ang II) induced arteriolar contractions under physiological conditions (Carlstrom et al., 2009, Just et al., 2007), and increased $\text{O}_2^{\cdot-}$ production in superoxide dismutase-1 knockout mice strengthens renal arteriolar contractions to Ang II (Carlstrom et al., 2010).

On the other hand, hydrogen peroxide (H_2O_2), which originates to some extent from catalytic reaction of $\text{O}_2^{\cdot-}$, also modulates vasomotor tone (Wolin et al., 2002). Interestingly, H_2O_2 can either enhance or impair vascular contractility dependent on its concentration (Chen et al., 2003, Lucchesi et al., 2005, Chen et al., 2007), but is thought to diminish vascular reactivity at physiological concentrations. We have shown recently that low concentrations of H_2O_2 impair myogenic tone in renal afferent arterioles, and that $\text{O}_2^{\cdot-}$ metabolism by superoxide dismutase generates sufficient H_2O_2 to oppose the $\text{O}_2^{\cdot-}$ -induced contraction (Li et al., 2016).

Ang II is an important renal vasoconstrictor and interacts with ROS to modulate renal arteriolar tone mainly via angiotensin type-1 receptors ($\text{AT}_1\text{-Rs}$) (Patzak et al., 2004, Patzak and Persson, 2007). Although the Ang II/ $\text{AT}_1\text{-Rs}$ signaling pathway contributes to IRI (Jang et al., 2014), its exact role is somewhat unclear. Renal ischemia is a dominant stimulus to renal renin release. The majority of investigations suggest an activation or a deleterious effect of the renin-Ang II-system (RAS) in IRI. Activation of RAS has been related to an Ang II mediated vasocontraction in IRI (Kontogiannis and Burns, 1998, Allred et al., 2000, da Silveira et al., 2010). However, there is also experimental evidence for a reduced vasoconstrictor response to Ang II immediately after ischemia (Conger and Weil, 1995).

Therefore, we tested the hypothesis that IRI-induced oxidative stress in renal afferent arterioles modulates Ang II elicited contraction focusing on $O_2^{\cdot-}$ and H_2O_2 .

A 45-minute bilateral renal ischemia was chosen to model severe IRI in humans, which can follow substantial ischemia time during vascular or cardiac surgery, aortic cross-clamping, cardiac arrest or shock, and affects both kidneys. The dose-response of isolated, perfused renal afferent arterioles to Ang II was investigated after IRI or sham operation. We related these functional responses to the levels of ROS in the arterioles. Thus, $O_2^{\cdot-}$ and H_2O_2 were measured in isolated vessels using fluorescence microscopy. The enzyme activity of superoxide dismutase and catalase was measured in pre-glomerular arterioles since the tissues from single dissected arterioles were insufficient for these assays. The functional contribution of $O_2^{\cdot-}$ and H_2O_2 was tested by incubating arterioles with PEG-SOD or PEG-catalase. The specificity of the Ang II related changes were investigated by using norepinephrine stimulated afferent arterioles, and the vascular specificity was addressed by using renal cortex tissue that primarily represents renal tubular epithelium.

Materials and Methods

Animals

Adult male C57Bl/6 mice (23~27 g) were maintained on rodent pelleted diet (0.4% of Na^+) and tap water, with 12h light/dark cycle. All the experiments were in accordance with the guidelines of the Animal Care and Ethical Committee of Zhejiang University School of Medicine.

Induction of renal IRI model

Mice were anesthetized with 4% chloral hydrate (6 mg/kg) intraperitoneal and IRI undertaken as described (Hu et al., 2014). Briefly, mice were infused with isotonic saline at a physiological temperature during the whole procedure. After visualizing both renal pedicles through a mid-line abdominal incision, the arteries of both kidneys were clamped individually with a non-traumatic microvascular clamp for 45 minutes. The renal ischemia was confirmed by blanching of kidneys. After ischemia, the clamps were released for the kidneys to start the reperfusion. Thereafter, the abdominal wound was sutured and the mouse was kept on a heating pad until it gains full consciousness. The mice were sacrificed after 24 hours of reperfusion and the kidneys were harvested. One kidney was sliced for isolation and perfusion of afferent arterioles and the other was used for measurements of $O_2^{\cdot-}$, H_2O_2 , SOD and catalase activity. Additional groups of mice were anesthetized to isolate preglomerular arterioles for SOD and catalase activity and RT-qPCR analysis.

Isolation and collection of renal preglomerular arterioles

As described previously (Chaudhari and Kirschenbaum, 1988, Lai et al., 2006, Guan et al., 2014), mice were anesthetized with 4% chloral hydrate (6 mg/kg i.p). The circulatory bed of the kidneys was isolated by ligating the abdominal aorta above the renal arteries and opening the aorta below to insert a catheter into the aortic lumen. Both kidneys were rinsed with physiologically buffered saline (PBS) and perfused with a solution of PBS plus 1% iron oxide and 1% albumin. After decapsulation, renal preglomerular arterioles were seen to be

filled with magnetized iron particles (Figure 1). The kidneys were removed, placed in ice-cold culture medium and chopped to very fine fragments. The minced renal tissue was passed through a 20-gauge needle (7 passages) and a 23-gauge needle (7 passages) to further dissociate the tissue. The vascular structures that were filled with magnetized iron particles were isolated from nonvascular tissue by attraction to a high-performance magnet. After resuspension in PBS solution, microvessels were separated on an 80 μm sieve and the quality of vessel checked by light microscopy. The procedure resulted in isolated microvessels free of glomeruli and tubules with a size $< 30 \mu\text{m}$ in diameter, which were used for the biochemical assays.

Isolation and microperfusion of afferent arterioles

Dissection and perfusion have been described previously (Patzak et al., 2004, Lai et al., 2006). Briefly, the afferent arterioles with their glomeruli intact from one kidney were carefully dissected by removing the connective tissues at 4°C in Dulbecco's modified Eagle's medium (DMEM) and transferred to a thermo-regulated chamber on the stage of an inverted microscopy. The time from dissection to perfusion was limited to less than 120 min. The afferent arterioles were perfused using glass micropipettes. Thereafter, the bath was gradually warmed to 37°C for 20 min before experiments. A pressure of 100 mmHg at the pressure head provided physiologic flow through the arterioles. Only arterioles that fully contracted to 100 mmol/L of KCl were used in the experiments.

Measurement of afferent arteriolar luminal diameter

The arteriolar luminal diameter was calculated from the mean of 5 consecutive pictures during the last 30 s of 2 min after each bolus application according to five different protocols (one protocol in one arteriole): 1. Bath addition of Ang II (10^{-12} – 10^{-7} mol/L). 2. Pre-incubation with 10 $\mu\text{mol/L}$ H_2O_2 followed by cumulative bath addition of Ang II. 3. Pre-incubation with PEG-catalase (1000 units/ml) (Li et al., 2015) followed by cumulative bath addition of Ang II. 4. Pre-incubation with PEG-SOD (200 units/ml) (Li et al., 2015) followed by cumulative bath addition of Ang II. 5. Cumulative bath addition of norepinephrine (10^{-12} – 10^{-4} mol/L).

Measurement of superoxide and hydrogen peroxide

As described previously (Li et al., 2015), arteriolar $\text{O}_2^{\cdot-}$ was assessed from PEG-SOD-inhibitable ethidium: dihydroethidium fluorescence ratio (E:DHE ; f/f_0) and H_2O_2 from PEG-catalase-inhibitable 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (C-H₂DCFDA) fluorescence. Renal cortical H_2O_2 and $\text{O}_2^{\cdot-}$ were analyzed using standard assays according to manufactures instructions (Kit for H_2O_2 from Beyotime Institute of Biotechnology, China, Kit for $\text{O}_2^{\cdot-}$ from Najing Jiancheng Bioengineering Institute, China). Briefly, the supernatants of samples were collected from homogenized renal cortex on ice by centrifugation at 12000 g for 3–5 min. Subsequently, the concentration of H_2O_2 was measured by incubation of supernatants with H_2O_2 test solutions for 30 min at room temperature and detection of the absorbance at 560 nm, $\text{O}_2^{\cdot-}$ level was determined by incubation of the sample with test solution at 37°C for 40 min and examination of the absorbance at 550 nm.

Enzymatic activity analysis

The supernatants from homogenized preglomerular arterioles, or from the renal cortex, were collected for measurements of catalase activity according to the manufacturer's instructions of catalase analysis kit (Beyotime Institute of Biotechnology, China). In this kit, metabolized H_2O_2 is coupled to a chromogenic substrate and treated with peroxidase to generate a red product (N-(4-antipyril)-3-chloro-5-sulfonate-p-benzoquinonemonoimine) that is quantitated in a spectrometer at a wavelength of 520 nm. One unit of catalase activity was defined as the amount of H_2O_2 metabolized per minute at room temperature. Catalase activity was determined by measuring the decomposition of H_2O_2 electrophotometrically. SOD activity levels were quantitated as a total superoxide dismutase by a kit (Beyotime, Shanghai) based on the chromogenic product of the WST-8. In this kit, water-soluble formazan dye is generated by WST-8 in the presence of superoxide anion driven by xanthine oxidase, detectable by a colorimetric assay, and quantitated in a spectrometer at 450 nm.

RT-qPCR analysis for AT₁R and AT₂R

Total RNA was extracted from renal preglomerular arterioles or from renal cortex by using TRIzol (Invitrogen) following the manufacturer's instructions. Thereafter, total RNA was reverse transcribed using random hexamers (Applied Biosystems). Real time quantitative PCR analysis was performed in 10 times reactions using the ABI7900 (Applied Biosystems) and SYBR GREEN PCR Master Mix (Applied Biosystems). A 245 bp length fragment of AT₁R gene and a 213 bp length fragment of AT₂R gene were amplified by the following primers: AT₁R forward: CAACTGCCTGAACCCTCTGT, AT₁R reverse: TATCTGAGGGCGGTAGGAG, AT₂R forward: CCTGGCAAGCATCTTATGTAGTTC, AT₂R reverse: CCGGAAATAAAATGTTGGCAAT. Expression of AT₁R and AT₂R gene was normalized relative to β -actin probes.

Chemical methods

Serum urea nitrogen (BUN) and creatinine were measured from the blood samples after renal ischemia and sham operations. Concentrations of BUN and creatinine were determined by specific enzyme urease and creatinine aminohydrolase, respectively. Urease converts urea to ammonia and carbonic acid. Ammonia is also generated when creatinine is hydrolyzed by creatinine aminohydrolase. These products, which are proportional to the concentrations of urea or creatinine in the sample, are assayed on the Roche Cobas C311 Analyzer (Diamond Diagnostics Inc., Holliston, MA).

Reagents

Dulbecco's modified Eagle's medium (DMEM) with low glucose from Gibco, USA was used for dissection, bath solution and perfusion. Ang II, polyethylene glycol catalase (PEG-CAT) and superoxide dismutase (PEG-SOD) were purchased from Sigma-Aldrich, St. Louis, MO. Hydrogen Peroxide Assay Kit, Catalase Analysis Kit and Total Superoxide Dismutase Assay Kit were purchased from Beyotime Institute of Biotechnology, China. Superoxide Anion Assay Kit was purchased from Jiancheng Bioengineering Company, Nanjing, China.

Statistics

All the results are presented as mean $\pm$ SEM. An analysis of variance with repeated measurements (ANOVA) was used to test concentration-dependent changes in afferent arteriolar diameter and to assess the differences in the afferent arteriolar responses between IRI and sham groups and the differences in the responses between groups of vessels incubated with vehicle, PEG-SOD or PEG-catalase. $P < 0.05$ was accepted as statistically significant.

RESULTS

IRI increased serum creatinine (Scr) and blood urea nitrogen (BUN) 5-fold compared to sham-operated mice ($P < 0.01$) (Table 1). Further, IRI doubled $O_2^{\cdot-}$ ($P < 0.001$) and H_2O_2 was increased by 38% ($P < 0.001$) in afferent arterioles, and similar increases in $O_2^{\cdot-}$ of 64% ($P < 0.05$) and H_2O_2 of 30% ($P < 0.001$) were seen in renal cortex compared to sham-operated mice (Figure 2). SOD activity was reduced by 17% ($P < 0.01$) and catalase activity by 45% ($P < 0.01$) in preglomerular arterioles from mice after IRI compared to sham-operated mice, which was also evident in the renal cortex (SOD -49%, $P < 0.001$ and catalase -48%, $P < 0.01$, Figure 3).

Compared to sham-operated mice, the basal luminal diameters of afferent arterioles were slightly larger in mice after IRI (11.4 ± 0.1 versus 10.0 ± 0.5 μm , $P < 0.05$) (Table 2). Incubation of normal arterioles with H_2O_2 led to a slowly developing vasodilation in arterioles without pre-constriction (Figure 4).

Ang II at concentration of 10^{-9} mol/L and above induced dose-dependent contraction of afferent arterioles from sham-operated mice that was reduced significantly in the afferent arterioles from mice after IRI (at 10^{-9} mol/L, $-5 \pm 3\%$ versus $-26 \pm 1\%$, $P < 0.001$; at 10^{-7} mol/L, $-27 \pm 2\%$ versus $-42 \pm 1\%$, $P < 0.001$). Pre-incubation of arterioles from sham-operated mice with H_2O_2 (10^{-5} mol/L for 15 min) prevented Ang II contractions at 10^{-9} and 10^{-8} mol/L and blunted the responses to Ang II at 10^{-7} mol/L (-22 ± 2 versus $-42 \pm 1\%$, $P < 0.001$) (Figure 5A). Pre-incubation of vessels from sham-operated mice with PEG-catalase (1000 units/ml for 30 min) did not affect the Ang II responses but increased the Ang II responses of arterioles from mice after IRI, which then contracted more than sham arterioles ($-50 \pm 4\%$ versus $-42 \pm 1\%$, $P < 0.05$) (Figure 5B). Pre-incubation of arterioles from sham-operated mice with 200 units/ml of PEG-SOD blunted the responses to Ang II ($-31 \pm 6\%$ versus $-42 \pm 1\%$, $P < 0.05$) but did not change the response to Ang II in vessels from mice after IRI (Figure 5C).

The dose response of afferent arterioles to norepinephrine was unchanged by IRI (Figure 6).

AT₁ and AT₂ receptor mRNA expressions were reduced after IRI both in preglomerular vessels and kidney cortex (Figure 7).

DISCUSSION

The main finding of the present study is that renal arteriolar H_2O_2 can buffer Ang II-induced contraction and may protect RBF after IRI. Isolated afferent arterioles from mice after IRI

had a selective reduction in their response to Ang II while the vasocontraction to norepinephrine was maintained. These arterioles had increased levels of H_2O_2 which were associated with decreased catalase activity. Further, exogenous H_2O_2 mimicked the effects of IRI on afferent arterioles from sham-operated mice, and incubation of arterioles from mice after IRI with PEG-catalase restored AngII elicited vasocontraction despite of reduced AT_1R mRNA expression. The observation of impaired Ang II responses of individual afferent arterioles after IRI is in agreement with previous reports of reduced renal microvascular reactivity after IRI and in others models of acute kidney injury (Wilkes et al., 1981, Guan et al., 2006).

Hypoxia and ischemia as well as reperfusion together with re-oxygenation are common in seriously ill patients. Both can induce large increases in renal and vascular ROS production (Wilcox, 2010, Granger and Kvietys, 2015). We confirmed significant increases in $\text{O}_2^{\cdot-}$ and H_2O_2 in isolated arterioles and renal cortical tissue after IRI. The main source of cellular H_2O_2 is the dismutation of $\text{O}_2^{\cdot-}$ by the superoxide dismutase (SOD) in the cell cytoplasm, mitochondria or extracellular space (Li et al., 2015). The concentration of H_2O_2 is also regulated by metabolism by various peroxidases of which catalase is most important in small blood vessels. It has recently been shown that H_2O_2 can be produced by mitochondrial monoamine oxidase, a source that was not explicitly ruled out in this study (Edmondson, 2014). H_2O_2 in afferent arterioles and renal cortex is thought to be derived from $\text{O}_2^{\cdot-}$. However, this production is likely limited by the observed suppression of SOD activity even though $\text{O}_2^{\cdot-}$ production is increased. More likely, the high levels of H_2O_2 relate to decreased metabolism of H_2O_2 by the reduced activity of catalase. H_2O_2 has a good diffusibility in tissues and therefore originates not only from vascular smooth muscle cells, but also from endothelium and the surrounding tubules. Therefore, we cannot exclude that the *in vivo* situation differs in regard to the effective H_2O_2 concentrations in the vessels and consequently *in vivo* effects on the vasculature may also differ. However, our data show similar depression of catalase activity and enhancement of H_2O_2 concentrations in both isolated vessels and renal cortical tissue. H_2O_2 can exert both vasoconstrictor (Gao and Lee, 2005) and vasodilator (Chen et al., 2007) effects depending on experimental conditions and the concentrations of H_2O_2 (Ardanaz and Pagano, 2006). We demonstrated that H_2O_2 caused a slow but substantial dilation of afferent arterioles and reduced the response to Ang II, consistent with results in mesenteric vessels (Prysyazhna et al., 2012). The mechanism behind the H_2O_2 -induced relaxation of renal afferent arterioles is not known. However, afferent arterioles express a wide range of membrane K^+ channels (Carlstrom et al., 2015) that can be activated by H_2O_2 , which would hyperpolarize the vascular smooth muscle cell membrane and may be an explanation (Rodriguez-Martinez et al., 1998, Matoba et al., 2000). These findings are consistent with a role for H_2O_2 as an endothelium-derived hyperpolarization factor (Shimokawa and Morikawa, 2005) and as the agent largely responsible for the reduced afferent arteriolar reactivity to Ang II in vessels from mice after IRI in this study.

Incubation of normal arterioles with H_2O_2 mimicked the effects of IRI. While incubation with PEG-catalase led to a substantial increase in arteriolar responses to Ang II in mice after IRI, the pre-incubation with PEG-SOD did not affect arteriolar responses to Ang II in mice after IRI. This again supports the hypothesis that reduced catalase activity is important for

increased H_2O_2 concentration. Interestingly, it appears that $\text{O}_2^{\cdot-}$ does not play an important role for the modulation of arteriolar reactivity in this model of IRI. This result is compatible with our recent report that incubation of afferent arterioles with H_2O_2 prevents the enhanced myogenic contraction by $\text{O}_2^{\cdot-}$ (Li et al., 2016). Indeed, incubation of arterioles from mice after IRI with PEG-catalase uncovered an enhanced responsiveness to Ang II that might represent the effects of the high levels of $\text{O}_2^{\cdot-}$.

Arteriolar responses to Ang II were impaired although responses to norepinephrine remained intact in mice after IRI. This extends studies on interlobular arteries in the mouse model of hypoxia/re-oxygenation where the response to Ang II was impaired, but the responses to norepinephrine or endothelin were unchanged (Kaufmann et al., 2015), similar to the effects of Ang II after IRI to selectively impair the renal vascular effect of Ang II (Guan et al., 2006). These findings indicate a specific modulation of the Ang II system and/or modified interaction of the Ang II induced signaling pathways with hypoxia/re-oxygenation induced signaling such as ROS induced or mediated pathways.

The reduced arteriolar expression of AT_1 receptor mRNA in mice after IRI provides another pathway that may contribute to the reduced Ang II responses. This observation extends several other studies that have reported a decreased AT_1 receptor mRNA expression after IRI (Kontogiannis and Burns, 1998, da Silveira et al., 2010). However, application of PEG-catalase more than reversed the diminished reactivity to Ang II after IRI despite the reduced AT_1 receptor expression, suggesting that AT_1 receptors may be post transcriptionally regulated in IRI. Whereas AT_1 receptors mediate most of the biological functions of Ang II including vasoconstrictions (Harrison-Bernard et al., 2003), the AT_2 receptor has little effect unless the AT_1 receptor is blocked or deleted after which AT_2 receptors can mediate vasodilation (Arendshorst et al., 1999, Duke et al., 2005). Thus, it is unlikely that the reduced mRNA expression of the AT_2 receptors in afferent arterioles from mice after IRI contributes to reduced responsiveness to Ang II.

CONCLUSION

Increased arteriolar levels of H_2O_2 impair afferent arteriolar contraction to Ang II in mice after IRI. This may be a protective effect in IRI since Ang II-mediated afferent vasoconstriction would impair GFR and could enhance renal parenchymal damage. The effect of H_2O_2 is relatively specific for Ang II, which indicates selective sensitivity of the Ang II system to H_2O_2 and/or the existence of shared and interacting signaling pathways for Ang II and H_2O_2 actions on arteriolar tone. The increased H_2O_2 concentration in renal arteriole and renal cortical tissue is related to a reduced catalase activity by IRI. This suggests that strategies to reduce H_2O_2 by antioxidant therapy must be evaluated carefully since increased H_2O_2 may be protective in the post-ischemic kidney (Wilcox, 2010).

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Figure 1.
Preglomerular arterioles in kidney filled with magnetized iron particles.

Figure 2.

Fluorescence signals for H_2O_2 and $O_2^{\cdot-}$ in perfused afferent arterioles (Panel A and B) or concentrations of H_2O_2 and $O_2^{\cdot-}$ in renal cortex (Panel C and D) from sham (open boxes) and IRI group (solid boxes). Mean $\pm$ SEM (n=6 per group), comparing groups: *, $P<0.05$; ‡, $P<0.001$.

Figure 3.

Catalase and SOD activity in preglomerular arterioles (Panel A and B) or in renal cortex (Panel C and D) from sham (open boxes) and IRI group (solid boxes). Mean $\pm$ SEM (n=6 per group), comparing groups: †, $P < 0.01$; ‡, $P < 0.001$.

Figure 4.
Time course of changes in luminal diameter of perfused normal afferent arterioles with 25 μM of H_2O_2 .

Figure 5.

Changes in luminal diameters of afferent arterioles in response to cumulative application of Ang II from sham (open circles with solid line), IRI (solid circles with broken line), sham pre-incubated with H₂O₂ (10 μ M) (cross-circles with solid line) or with PEG-catalase (1000 units/ml) (open squares with solid line) or with PEG-SOD (200 units/ml) (open triangles with solid line), and IRI pre-incubated with PEG-catalase (solid squares with broken line) or with PEG-SOD (solid triangles with broken line). Mean $\pm$ SEM (n=6 per group).

Figure 6.

Changes in luminal diameters of afferent arterioles in response to cumulative application of norepinephrine from sham (open circles with solid line) and IRI group (solid circles with broken line). Mean $\pm$ SEM (n=6 per group), comparing groups: p=NS.

Figure 7.

Expression of mRNAs for AT₁R and AT₂R in preglomerular arterioles (Panel A) or renal cortex (Panel B) from sham (open circles) and IRI group (solid circles). Mean ± SEM (n=8 per group), comparing groups: *, $P < 0.05$; ‡, $P < 0.001$.

Table 1

Serum creatinine and blood urea nitrogen

	Sham	IRI
Serum creatinine (nmol/L)	22.3 ± 0.3	101.3 ± 0.8 [‡]
Blood urea nitrogen (nmol/L)	12.1 ± 0.3	59.4 ± 1.3 [‡]

Mean ± SEM (n=5–6 per group) in mice with sham or 24 hours after ischemia reperfusion injury (IRI). Compared to sham group:

[‡]*P*<0.01.

Table 2

Basal afferent arteriolar diameter

	Arteriolar diameter (μm)	
	Sham	IRI
Vehicle	10.0 $\pm$ 0.5	11.4 $\pm$ 0.1 *
PEG-SOD	10.7 $\pm$ 0.5	10.9 $\pm$ 0.5
PEG-CAT	10.9 $\pm$ 0.6	11.8 $\pm$ 0.3

Mean $\pm$ SEM (n=5–6 per group) in mice with sham or 24 hours after ischemia reperfusion injury (IRI), 30 min after bath addition of PEG-SOD (200 units/ml) or PEG-CAT (1000 units/ml) Compared to sham group:

* P<0.05.