Superoxide and hydrogen peroxide counterregulate myogenic contractions in renal afferent arterioles from a mouse model of chronic kidney disease

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Myogenic contractions protect kidneys from barotrauma but are impaired in chronic kidney disease (CKD). Since myogenic contractions are enhanced by superoxide but impaired by hydrogen peroxide, we tested the hypothesis that they are counterregulated by superoxide and H_2O_2 from NOX2/p47phox and/or NOX4/POLDIP2 in CKD. Myogenic contraction in isolated perfused afferent arterioles from mice with surgical 5/6 nephrectomy or sham operations fed a 6% sodium chloride diet was measured directly while superoxide and H_2O_2 were measured by fluorescence microscopy. Compared to sham-operated animals, an increase in perfusion pressure of arterioles from CKD mice doubled superoxide (21 versus 11%), increased H_2O_2 sevenfold (29 versus 4%), and reduced myogenic contractions profoundly (-1 versus -14%). Myogenic contractions were impaired further by PEG-superoxide dismutase or in arterioles from p47phox-/- (versus wild type) mice but became supra-normal by PEG-catalase or in mice with transgenic expression of catalase in vascular smooth muscle cells (-11 versus -1%). Single arterioles from mice with CKD expressed over 40% more mRNA and protein for NOX4 and POLDIP2. Myogenic responses in arterioles from POLDIP2 +/- (versus wild type) mice with CKD had over an 85% reduction in H_2O_2, but preserved superoxide and a normal myogenic response. Tempol administration to CKD mice for 3 months decreased afferent arteriolar superoxide and H_2O_2 and maintained myogenic contractions. Thus, afferent arteriolar superoxide generated by NOX2/p47phox opposes H_2O_2 generated by NOX4/POLDIP2 whose upregulation in afferent arterioles from mice with CKD accounts for impaired myogenic contractions.

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Received 30 November 2016; revised 23 January 2017; accepted 9 February 2017; published online 7 April 2017

Kidney International (2017) **92,** 625–633; http://dx.doi.org/10.1016/j.kint.2017.02.009

KEYWORDS: oxidative stress; reduced renal mass; renal autoregulation; tempol

Chronic kidney disease (CKD) can be modeled experimentally by surgically reduced renal mass (RRM).[1] The progression of CKD remains poorly understood but has been related to reactive oxygen species (ROS),[2,3] hypertension,[4] and impaired renal autoregulation.[5] A combination of hypertension and impaired autoregulation can increase the pressure in the glomerular capillaries and renal parenchyma and lead to barotrauma that accelerates the loss of kidney function.[1,4,5] Renal autoregulation is mediated by the tubuloglomerular feedback response but primarily by a rapid myogenic contraction of the vascular smooth muscle cells of the afferent arteriole in response to an increase in perfusion pressure.[6,7] Therefore, this study focused on the mechanisms of impaired myogenic responses of afferent arterioles from the kidneys in a model of CKD.

An increase in perfusion pressure of individual afferent arterioles isolated from normal mouse kidneys increases vascular superoxide ($O_2^{\cdot-}$) from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[8] that mediates much of the normal myogenic contraction.[8–10] However, interventions that generate ROS are reported to have variable effects on myogenic contractions and autoregulation. Thus, afferent arterioles from spontaneously hypertensive rats have exaggerated myogenic contractions that are dependent on $O_2^{\cdot-}$ from NADPH oxidase.[11] However, metabolism of ROS by tempol paradoxically increases the myogenic contractions of afferent arterioles from rats with oxidative stress from transforming growth factor-β.[12] Moreover, renal autoregulation and myogenic responses of afferent arterioles are impaired in mice with RRM[13] despite considerable oxidative stress.[3] We reported recently that exogenous H_2O_2 dose-dependently impaired myogenic contractions and blunted

the effects of $O_2^{\cdot-}$ in normal mouse afferent arterioles.[10] However, H_2O_2 was not produced by increasing the perfusion pressure of normal arterioles.

The renal afferent arterioles of normal mice express the genes for $p22^{phox}$ that act primarily to direct neutrophil oxidase (NOX) to the cell membrane. NOX2 with its cytoplasmic activator $p47^{phox}$ is expressed in renal afferent arterioles. Arterioles also express NOX4 and its regulator POLDIP2 (polymerase [DNA-directed] delta-interacting protein 2).[14,15] NOX1 is produced primarily in larger vessels, and NOX5 is not expressed in rodents. We have reported that $p47^{phox\ -/-}$ mice generate less $O_2^{\cdot-}$ in their afferent arterioles and have enfeebled myogenic contractions.[8] However, NOX4 may generate H_2O_2 directly,[14] but its effects on afferent arterioles are not known.

C57BL/6 mice with RRM have an adaptive increase in glomerular filtration rate of residual nephrons but develop only modest glomerular damage or tubulointerstitial fibrosis, likely because of the lack of hypertension. However, these mice have severely impaired myogenic contractions of their afferent arterioles.[13] Thus, this is a convenient model with which to study the functional adaptation to RRM without the complications of uremia, hypertension, or extensive glomerulosclerosis and arteriolar damage. The RRM mouse model of CKD has a 6-fold increase in $O_2^{\cdot-}$, as indicated by 8-isoprostane excretion, a tripling of H_2O_2 excretion, and a 28% reduction in myogenic responses.[3] A high salt diet for 3 months did not significantly affect the myogenic responses of normal mice yet increased 8-isoprostane and H_2O_2 excretion and renal NADPH oxidase activity[16] and further worsened myogenic contractions in afferent arterioles from mice with RRM.[13] Thus, NaCl in the diet does not itself regulate myogenic contractions but enhances the effects of RRM. Therefore, mice were fed a 6% NaCl diet to further enhance the oxidative stress and defective myogenic responses associated with RRM. Myogenic contractions were enhanced at 3 days after RRM, but were reduced at 3 weeks and were absent at 3 months.[13] Therefore, this study was limited to mice 3 months after RRM or sham operation. To test the hypothesis that H_2O_2 from NOX4 opposes NOX2-derived, $O_2^{\cdot-}$-induced myogenic contraction, afferent arterioles from $p47^{phox}$ or *POLDIP2* gene deleted mice were used. To test the hypothesis that reduced myogenic contractions in mice with RRM derived H_2O_2, afferent arterioles from mice with vascular smooth muscle cell transgenic for catalase ($Tg^{cat\text{-}SMC}$) were used. Tempol was administered for 3 months to groups of wild-type mice to normalize excessive excretion of 8-isoprostane and H_2O_2 in those with RRM.[3] These studies have potential clinical impact because several antioxidant strategies are under investigation yet none is targeted to a specific ROS or NOX isoform.

RESULTS

The figures represent myogenic and fluorescence data for arterioles perfused at 40 and 80 mm Hg, whereas Table 1 shows basal data at 40 mm Hg and the myogenic response that was calculated by the slope of the regression of active wall tension on perfusion pressure across the full range of perfusion pressure from 40 to 134 mm Hg because the relationship was linear across this range.

Impaired myogenic contractions of afferent arterioles from mice with RRM and a high salt diet can be ascribed to excessive H_2O_2 rather than $O_2^{\cdot-}$

The basal diameters of afferent arterioles perfused at 40 mm Hg differed among groups (Table 1). Therefore, we presented the percentage of change in arteriolar luminal diameter. Increasing perfusion pressure of afferent arterioles from sham-operated mice from 40 to 80 mm Hg reduced the luminal diameter substantially ($-14 \pm 4\%$) (Figure 1). In contrast, afferent arterioles from mice after RRM did not contract significantly with increasing perfusion pressure from 40 to 80 mm Hg ($-1 \pm 4\%$) (Figure 1).

Despite severely impaired myogenic contractions, arterioles from mice with RRM generated twice as much of $O_2^{\cdot-}$ with increased perfusion pressure from 40 to 80 mm Hg (RRM: $21 \pm 2\%$ vs. sham: $11 \pm 3\%$; $P < 0.01$) (Figure 1a). Incubation with polyethylene glycol–superoxide dismutase (PEG-SOD) to metabolize $O_2^{\cdot-}$ reduced myogenic contractions of arterioles from both sham and RRM groups (Figure 1b). An increase in perfusion pressure of arterioles from mice with RRM incubated with PEG-SOD paradoxically increased their diameter (RRM + PEG-SOD: $+3 \pm 1\%$ vs. RRM: $-1 \pm 4\%$, $P < 0.05$) (Figure 1b). Genetic deletion of $p47^{phox}$ largely prevented arteriolar $O_2^{\cdot-}$ generation (Figure 1c) and had similar effects as incubation with PEG-SOD on impairing myogenic contractions (RRM + $p47^{Phox}$ $-/-$: $+2 \pm 1\%$ vs. RRM: $-1 \pm 4\%$, $P < 0.05$) (Figure 1d).

Table 1 | Basal afferent arteriolar diameter and myogenic responses 3 months after reduced renal mass or sham operations in mice given vehicle or oral tempol

Group	N	Basal diameter for myogenic studies (μm)	Myogenic response (dynes·cm^{-1}·mm Hg^{-1})
WT sham + vehicle	5	10.4 ± 0.6	3.90 ± 0.38
WT RRM + vehicle	7	10.0 ± 1.0	1.63 ± 0.25[a]
WT sham + tempol	5	10.2 ± 0.6	2.70 ± 0.28[b]
WT RRM + tempol	7	9.0 ± 0.4	2.22 ± 0.19
WT sham + PEG-SOD	5	6.2 ± 0.6	1.43 ± 0.17[a]
WT sham + PEG-catalase	6	10.6 ± 1.1	3.0 ± 0.21
WT RRM + PEG-SOD	4	9.0 ± 2.2	2.07 ± 0.78
WT RRM + PEG-catalase	7	11.1 ± 1.0	3.83 ± 0.27[c]
$p47^{Phox-/-}$ RRM	5	10.0 ± 1.3	1.03 ± 0.38
$Tg^{Cat\text{-}SMC}$ RRM	5	8.6 ± 0.6	3.05 ± 0.36[c]
POLDIP2+/− sham	5	8.5 ± 0.6	2.69 ± 0.28[b]
POLDIP2+/− RRM	7	11.1 ± 0.6	2.87 ± 0.12[c]

PEG-SOD, polyethylene glycol–superoxide dismutase; RRM, reduced renal mass; $Tg^{Cat\text{-}SMC}$, vascular smooth muscle cell transgenic for catalase; WT, wild-type.
Values shown are mean ± SEM. Basal diameter was measured at 40-mm Hg perfusion pressure. Myogenic response was calculated as the slope of regression of active wall tension on perfusion pressure.
Compared with WT sham: [a]$P < 0.005$ and [b]$P < 0.05$ compared with WT RRM, [c]$P < 0.05$.