Enhanced Superoxide Production in Renal Outer Medulla of Dahl Salt-Sensitive Rats Reduces Nitric Oxide Tubular-Vascular Cross-Talk

Takefumi Mori, Paul M. O’Connor, Michiaki Abe, Allen W. Cowley, Jr

Abstract—Studies were conducted to determine whether the diffusion of NO from the renal medullary thick ascending limb (mTAL) to the contractile pericytes of surrounding vasa recta was reduced and, conversely, whether diffusion of oxygen free radicals was enhanced in the salt-sensitive Dahl S rat (SS/Mcwi). Angiotensin II ([Ang II] 1 μmol/L)–stimulated NO and superoxide (O$_2$\textsuperscript{−}) production were imaged by fluorescence microscopy in thin tissue strips from the inner stripe of the outer medulla. In prehypertensive SS/Mcwi rats and a genetically designed salt-resistant control strain (consomic SS-13BN), Ang II failed to increase either NO or O$_2$\textsuperscript{−} in pericytes of isolated vasa recta. Ang II stimulation resulted in production of NO in epithelial cells of the mTAL that diffused to vasa recta pericytes of SS-13BN rats but not in SS/Mcwi rats except when tissues were preincubated with the superoxide scavenger TIRON (1 mmol/L). Ang II resulted in a greater increase of O$_2$\textsuperscript{−} in the mTAL of SS/Mcwi compared with SS-13BN mTAL. The O$_2$\textsuperscript{−} diffused to adjoining pericytes in tissue strips only in SS/Mcwi rats but not in control SS-13BN rats. Diffusion of Ang II–stimulated O$_2$\textsuperscript{−} from mTAL to vasa recta pericytes was absent when tissue strips from SS/Mcwi rats were treated with the NO donor DETA-NONOate (20 μmol/L). We conclude that the SS/Mcwi rat exhibits increased production of O$_2$\textsuperscript{−} in mTAL that diffuses to surrounding vasa recta and attenuates NO cross-talk. Diffusion of O$_2$\textsuperscript{−} from mTAL to surrounding tissue could contribute to reduced bioavailability of NO, reductions of medullary blood flow, and interstitial fibrosis in the outer medulla of SS/Mcwi rats. (Hypertension. 2007;49:1336-1341.)

Key Words: Dahl rat ▪ NO ▪ superoxide ▪ renal medulla ▪ cross-talk

There is evidence that Dahl salt-sensitive rats exhibit increased renal medullary oxidative stress compared with Dahl salt-resistant rats, and when treated with the superoxide dismutase mimetic TEMPOL, they exhibit less salt-induced hypertension and renal damage. Recently, superoxide (O$_2$\textsuperscript{−}) production in the outer medullary region of the Medical College of Wisconsin inbred SS (SS/Mcwi) rat was found to be significantly higher when compared with a genetically designed, salt-resistant control strain, the consomic SS-13BN. This was evident even when rats were studied while on a 0.4% salt diet in a prehypertensive state. Interestingly, no differences in the tissue levels of NO and NO synthase activities were observed between these 2 rat strains.

Other studies have provided evidence that there is diffusion of O$_2$\textsuperscript{−} and NO radicals between the tubules of the medullary thick ascending limb (mTAL) and the surrounding vasa recta vasculature of the outer medulla, which we have referred to as “tubulovascular cross-talk.” In vitro studies using real-time fluorescent imaging of isolated thin tissue strips from the outer medulla have also demonstrated that O$_2$\textsuperscript{−} can diffuse from the mTAL to the surrounding vasa recta pericytes in Sprague–Dawley rats. This was found to be the case, however, only when NO tissue levels were scavenged with carboxy-PTIO. It was also found that tubulovascular NO cross-talk was reduced under conditions of increased production of O$_2$\textsuperscript{−} in mTAL and that diffusion of NO from mTAL to pericytes was enhanced by reduction of O$_2$\textsuperscript{−} in this region. These observations are consistent with studies by Ortiz and Garvin, who demonstrated in cortical TAL that O$_2$\textsuperscript{−} scavenged with TEMPOL (a cell-permeable O$_2$\textsuperscript{−} dismutase mimetic) increased arginine-induced NO release. Together, these studies demonstrated that a homeostatic balance between NO and O$_2$\textsuperscript{−} production is required under normal conditions. An exaggerated response of either NO or O$_2$\textsuperscript{−} would be expected if the response of the counteracting system was suppressed either pharmacologically or naturally.

We hypothesized that because elevated tissue levels of O$_2$\textsuperscript{−} occur naturally in the SS/Mcwi rat in contrast to the Sprague–Dawley or other salt-insensitive control rats, they would exhibit an exaggerated mTAL production and diffusion of O$_2$\textsuperscript{−} to the surrounding vasa recta pericytes in response to angiotensin II (Ang II). Studies were, therefore, carried out using thin tissue strips isolated from the renal outer medulla...

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of SS/Mcwi and control consomic SS-13<sup>BN</sup> rats to determine the effects of Ang II on intracellular NO and O<sub>2</sub>⁻ production of mTAL epithelia and vasa recta pericytes using 4,5-diaminofluorescein diacetate (DAF-2DA) and dihydro-ethidium (DHE) with dynamic fluorescence imaging techniques.<sup>7,8,10,11</sup> Responses of isolated mTAL alone and pericytes of isolated vasa recta were first determined. Then, to determine the degree of tubulovascular cross-talk of both O<sub>2</sub>⁻ and NO in pericytes, tissue strips containing mTAL surrounded by vasa recta were studied. The results of these studies show that the prehypertensive SS/Mcwi rats fed a 0.4% salt diet exhibit increased mTAL O<sub>2</sub>⁻ production in response to Ang II stimulation and a greater cross-talk of this free radical to surrounding vasa recta pericytes compared with the consomic SS-13<sup>BN</sup> control strain.

**Methods**

**Determination of Dye Specificities**

Dye specificities of DAF-2DA and DHE were determined in vitro by comparing responses to changes of NO, O<sub>2</sub>⁻, peroxynitrite, and hydrogen peroxide concentrations using doses of generators expected to produce levels slightly above physiological reported concentrations.<sup>10,15</sup> The non–cell-permeable forms of DAF-2 (40 μmol/L) and DHE (50 μmol/L) were incubated for 30 minutes at a final volume of 1 mL at 37°C with the following solutions: (1) low NO (100 μmol/L of NO donor diethylpentamine NONOate [DETA-NONOate], Cayman); (2) high NO 1 mmol/L of DETA-NONOate; (3) low O<sub>2</sub>⁻: (25 μmol/L, Sigma) with 3 μmol/L of xanthine oxidase as a O<sub>2</sub>⁻ generator<sup>13</sup> (Sigma); (4) high O<sub>2</sub>⁻ (pterin: 25 μmol/L) with 28 μmol/L of xanthine oxidase; (5) peroxynitrite with excess O<sub>2</sub>⁻ (low NO with high O<sub>2</sub>⁻); (6) peroxynitrite with excess NO (high NO with low O<sub>2</sub>⁻); and (7) hydrogen peroxide solution (1 mmol/L) for DHE (167 μg/mL of salmon DNA was added to enhance the signal; coproduction of NO and O<sub>2</sub>⁻ with combination of DETA-NONOate and pterin with xanthine oxidase produces peroxynitrite).<sup>14</sup> Incubated solutions were formed in a KIMBLE glass capillary (VWR Scientific), and images were captured with a Nikon E600 fluorescence microscope (Flyer Co) equipped with a cooled charge-coupled device camera (Princeton Instruments). The intensity of the images was analyzed with Meta-morph image analysis software (Universal Imaging).

**Animal Use and Preparation of Tissues for Fluorescence Imaging**

Using techniques that we have published previously,<sup>7,8,10</sup> renal microtissue strips were dissected from the outer medulla of the left kidney of male, pentobarbital-anesthetized (60 mg/kg IP) inbred Dahl salt-sensitive rats (Dahl SS/JrHsdMcwi; SS/Mcwi) and consomic SS-13<sup>BN</sup> control rats (SS-13<sup>BN</sup>/Mcwi; SS-13<sup>BN</sup>),<sup>4</sup> a strain that is 98% genetically identical to the SS/Mcwi strain but is largely protected from salt-induced hypertension.<sup>15,16</sup> Rats 8 to 9 weeks of age weighing 170 to 230 g were fed a 0.4% salt diet (Dyets Inc) since weaning. Left kidneys were cleared of blood by perfusing with Hank’s Balanced Salt Solution (Life Technologies) containing 100 mmol/L of HEPES and 1 mg/mL of BSA (HBSSH; Sigma, adjusted to pH 7.4). To image the pericytes surrounding the endothelial cells of the vasa recta, latex microspheres in solution (2.7% weight/vol; 0.2 μm in diameter; Polysciences) were infused into the kidneys before microdissection of the tissue strips to denude the endothelium and remove the fluorescent signals underlying the pericytes.<sup>7,8</sup> Fluorescence imaging of the thin tissue strips mounted on coverslips was done using techniques that we have described in detail previously.<sup>7,8,10,11</sup> L-Arginine (AG; 100 μmol/L, Sigma) was added to HBSSH (HBSSH-AG) to maintain physiological NO production during the measurement of intracellular O<sub>2</sub>⁻ and NO. The coverslips were loaded with either DHE (50 mmol/L in HBSSH-AG; Molecular Probes) for intracellular O<sub>2</sub>⁻ measurement or DAF-2DA (10 μmol/L in HBSSH-AG; Calbiochem-Novabiochem) for intracellular NO measurement and suffused for 1 hour at room temperature. The coverslip-mounted tissue strip was placed in a temperature-controlled imaging chamber system (RC-40, Warner Instruments Inc) and covered with an 18-mm round coverslip before the 200 μL/min suffusion of the buffer solution at 37°C constant temperature.

**Tissue Protocols**

The excess dye from the isolated tissue strips was washed 3 times with HBSSH-AG after loading and incubated for 30 minutes in 1 of 2 solutions: HBSSH-AG alone or HBSSH-AG with 1 mmol/L of O<sub>2</sub>⁻ scavenger, 4,5-di-hydroxy-1,3-benzenediisulfonic acid (TIRON, Sigma). NO and O<sub>2</sub>⁻ responses were imaged in response to superfusion of the tissue strips first with the drug vehicle (HBSSH-AG) for 300 seconds followed by superfusion with Ang II (1 μmol/L in HBSSH-AG, Sigma). To test for dye loading and cell viability,<sup>7,8,10,11</sup> positive control stimuli were applied by following the Ang II responses with either DETA-NONOate (100 μmol/L for mTAL and 1 mmol/L for pericyte response; Cayman) for DAF-2DA–loaded tissue or 1 mmol/L of the Cu/Zn O<sub>2</sub>⁻ dismutase inhibitor diethyldithiocarbamic acid (Sigma) with 500 μmol/L of menadione sodium bisulfite (Sigma) added to stimulate mitochondrial O<sub>2</sub>⁻ release in DHE-loaded tissue.<sup>7,11</sup> All of the O<sub>2</sub>⁻ and NO responses were compared at a 300-second time period after each stimulus. Because differences in dye loading prevent direct comparison of raw fluorescence data between tissue strips, no attempt was made to compare absolute values of agonist responses between the strains. NO responses were normalized with baseline levels that were acquired >100 seconds before the vehicle responses, as described previously.<sup>17</sup> Ethidium (Eth)/DHE was not normalized to prevehicle baseline, because this was expressed as a ratio thereby removing raw signal intensity or loading effects. However, the Eth/DHE ratios were adjusted to ~1.0 at the beginning of each experimental protocol to enhance the detection sensitivity.

**Statistical Analysis**

Values are expressed as mean±SE. A paired <i>t</i> test was used to compare drug vehicle and agonist responses at 300 seconds after the stimulation. For comparison of the responses between the strains, Δ changes of Ang II response differences from vehicle treatment were compared using a nonpaired <i>t</i> test. Significance was accepted at a level of <i>P</i> < 0.05. All of the protocols were approved by the Institutional Animal Care Committee.

**Results**

**Specificity of the NO and O<sub>2</sub>⁻ Fluorescence Indicators**

Application of the NO donor DETA-NONOate at 2 concentrations (100 μmol/L and 1 mmol/L), increased DAF-2DA fluorescence in a dose-dependent manner (Table). No other
condition (excess $O_2^-$, peroxynitrite, or hydrogen peroxide) produced a change in DAF-2DA fluorescence indicating the specificity of DAF-2DA for NO over a broad physiological range. The generation of $O_2^-$ at 2 levels with the addition of pterin and xanthine oxidase increased Eth fluorescence in a dose-dependent manner (0.5 mM/L and 5 mM/L of xanthine oxidase). Conditions of excess NO, peroxynitrite, and hydrogen peroxide did not increase Eth fluorescence, suggesting that physiological levels of NO, peroxynitrite, and hydrogen peroxide do not oxidize DHE. These results show that the fluorescent indicators for NO and $O_2^-$ were specific within the limits of what could be assessed, indicating that the responses to Ang II within the tissues seen in the present study were because of either NO or $O_2^-$. $P<0.05$) when compared with vehicle responses. The pericytes of vasa recta adjacent to mTAL in tissue of SS/Mcw1 rats preincubated with the $O_2^-$ scavenger TIRON (1 mM/L) exhibited a significant rise of NO in response to Ang II (84.9±28.1 U; n=6; $P<0.05$) and in mTAL alone (57.2±17.5 U; n=5; $P<0.05$; Figure 1, middle). It was also found in SS.13BN rats that NO responses to Ang II within mTAL in the presence of TIRON were not significantly different from those responses of the SS/Mcw1 mTAL rats (N=5 rats; data not shown). Administration of Ang II also resulted in a significant increase in NO within the isolated mTAL tissue preparations from SS-13BN rats, whereas no response was seen in SS/Mcw1 rats (Figure 1, middle). Ang II responses were compared statistically with vehicle responses in all of the cases, and at the end of each experiment, the NO donor, DETA-NONOate (100 µmol/L for mTAL and 1 mM/L for pericyte response), was administered to test for dye saturation or cell death. The NO response to DETA-NONOate averaged 7- to 15-fold higher in pericytes and 8- to 20-fold higher in mTAL than the vehicle responses.

Responses were quite different when studied in pericytes of isolated vasa recta without surrounding mTAL (Figure 1, bottom). This same dose of Ang II failed to increase NO in SS/Mcw1 (2.7±10.6; n=6), SS-13BN (9.0±6.6; n=5), or SS/Mcw1 treated with TIRON (7.8±2.6; n=6) as compared with the vehicle responses. These results, when compared with those observed in pericytes of vasa recta that were adjacent to mTAL (Figure 1, top), indicate that $O_2^-$ inhibits NO diffusion from mTAL to adjacent vasa recta pericytes.

**Figure 1.** NO responses to Ang II in outer medullary tissue strips of SS and SS-13BN rats. Top, NO responses to either vehicle or to Ang II (1 µmol/L) in pericytes of endothelium-disrupted vasa recta adjacent to mTAL determined at 300 seconds after stimulation by Ang II in SS rats (■), SS-13BN (□), and SS with TIRON (○). Center, NO responses to the same dose of Ang II in outer medullary tissue strips containing epithelial cells of mTAL determined at 300 seconds after stimulation. Bottom, NO responses to the same dose of Ang II in pericytes of isolated endothelium-disrupted vasa recta determined at 300 seconds after stimulation. *P<0.05 significant from vehicle response. †P<0.05 significant from response in SS rats.

**NO Responses to Ang II of SS/Mcw1 and SS-13BN Rats**

As summarized in Figure 1, Ang II (1 µmol/L) significantly increased the Eth/DHE fluorescence ratio in pericytes of vasa recta of SS/Mcw1 rats that were adjacent to mTAL (0.26±0.05; n=8; $P<0.05$; Figure 2, top) and in mTAL alone (0.46±0.11; n=8; $P<0.05$; Figure 2, middle) of SS/Mcw1 rats compared with the vehicle response (0.15±0.02; n=8 and 0.22±0.04; n=8). In contrast, this response was not seen in pericytes of vasa recta adjacent to mTAL in SS-13BN rats (0.16±0.02; n=8) when compared with vehicle responses (0.15±0.04; n=8). A small but significant rise of the Eth/DHE fluorescence ratio was seen in isolated mTAL alone of SS-13BN rats although pericytes studied in the absence of surrounding mTAL (Figure 2, bottom) showed no increases of $O_2^-$ in response to Ang II in either the SS/Mcw1 or SS-13BN rats when compared with vehicle responses. In all of the preparations used in this analysis, positive Eth/DHE fluorescence control responses were obtained in response to 1 mM/L of diethyldithiocarbamic acid with menadione (500 µmol/L), indicating that dye saturation had not occurred and that the cells were viable. Taken together, these results indicate that diffusion of $O_2^-$ from mTAL to pericytes took place in the SS/Mcw1 rats but not in the control SS-13BN rat strain.

**$O_2^-$ Responses to Ang II in SS/Mcw1 and SS-13BN Rats**

As shown in Figure 2, Ang II (1 µmol/L) significantly increased the Eth/DHE fluorescence ratio in pericytes of vasa recta of SS/Mcw1 rats that were adjacent to mTAL (0.26±0.05; n=8; $P<0.05$; Figure 2, top) and in mTAL alone (0.46±0.11; n=8; $P<0.05$; Figure 2, middle) of SS/Mcw1 rats compared with the vehicle response (0.15±0.02; n=8 and 0.22±0.04; n=8). In contrast, this response was not seen in pericytes of vasa recta adjacent to mTAL in SS-13BN rats (0.16±0.02; n=8) when compared with vehicle responses (0.15±0.04; n=8). A small but significant rise of the Eth/DHE fluorescence ratio was seen in isolated mTAL alone of SS-13BN rats although pericytes studied in the absence of surrounding mTAL (Figure 2, bottom) showed no increases of $O_2^-$ in response to Ang II in either the SS/Mcw1 or SS-13BN rats when compared with vehicle responses. In all of the preparations used in this analysis, positive Eth/DHE fluorescence control responses were obtained in response to 1 mM/L of diethyldithiocarbamic acid with menadione (500 µmol/L), indicating that dye saturation had not occurred and that the cells were viable. Taken together, these results indicate that diffusion of $O_2^-$ from mTAL to pericytes took place in the SS/Mcw1 rats but not in the control SS-13BN rat strain.

To determine whether diffusion of $O_2^-$ in the outer medullary region of SS/Mcw1 rats would be reduced by raising tissue
Figure 2. O$_2^-$ responses to Ang II in outer medullary tissue strips of SS and SS-13BN rats. Changes (Δ) of normalized Eth/DHE responses over 300 seconds following either vehicle or Ang II stimulation are shown. A, O$_2^-$ responses to either vehicle or to Ang II (1 μmol/L) in pericytes of endothelium-disrupted vasa recta adjacent to mTAL determined at 300 seconds after stimulation. □, SS rats; □, SS-13BN. B, O$_2^-$ responses to same dose of Ang II in outer medullary tissue strips containing epithelial cells of mTAL determined at 300 seconds after stimulation. *P<0.05 significant from vehicle response. †P<0.05 significant from response without NO donor.

**Discussion**

Previous studies have found that Ang II resulted in increases of NO within the pericytes vasa recta and that the source of this NO was derived from surrounding mTAL. Ang II failed to increase pericyte or endothelial levels of NO in isolated vasa recta denuded of endothelial cells. It was also found that, although the Ca$^{2+}$ ionophore (A23187) increased endothelial NO concentrations, this ionophore failed to increase pericyte NO in isolated vasa recta. These results indicated that the machinery to couple Ang II to NO synthase was present in vasa recta endothelial cells but not in pericytes. In contrast, Ang II was found to increase both Ca$^{2+}$ concentrations and NO in isolated mTAL. Importantly, only when vasa recta were adjacent to mTAL in a tissue strip did the addition of Ang II increase pericyte NO levels. The relevance of these responses is based on microdialysis studies showing that both NO and O$_2^-$ diffuse into the interstitial fluid, and the concentration of these free radicals reciprocally determine the medullary blood flow.

**Reduced Diffusion of NO From mTAL to Vasa Recta Pericytes in the Outer Medullary Region of SS/Mcwi Rats**

The present study has found that, in SS/Mcwi rats, Ang II failed to increase NO levels in epithelial cells of isolated mTAL, and tubulovascular cross-talk was not seen in the pericytes of vasa recta that were adjacent to these mTAL. These responses were quite different in the SS-13BN strain in which NO was significantly increased in the mTAL by Ang II stimulation. The reduced NO production in the SS/Mcwi rat compared with the SS-13BN rats cannot be attributed to differences in medullary NO synthase enzyme activity or NO levels in the outer medulla, because it has been found that they do not differ significantly. NO synthase enzyme activity and protein levels of both the SS/Mcwi and SS-13BN, however, are significantly lower than that found in the parental BN rat strain. It is, therefore, likely that...
enhanced O$_{2}^-$ production in SS/Mcw1 rats resulted in reduced NO bioavailability within the medulla. Significant differences in baseline NO production between the SS/Mcw1 and SS-$13^{BN}$ were not detected; however, differences between these tissues were clearly observed in the Ang II–stimulated state (see Figure 3). Finally, treatment of tissue strips with 1 mmol/L of TIRON in the present study normalized the NO response to Ang II between the 2 strains (Figure 1), further indicating that elevated O$_{2}^-$ responses in SS/Mcw1 rats were responsible for the NO responses in SS/Mcw1 rats. Also, as indicated in the Results section, the mTAL NO response of SS.$13^{BN}$ rats to Ang II in the presence of TIRON did not differ from that of the SS/Mcw1 rats.

**Diffusion and Interactions of O$_{2}^-$ Radicals in the Renal Outer Medullary Region of SS Rats**

The results of this study show that mTAL of SS/Mcw1 rats produce greater amounts of O$_{2}^-$ in response to Ang II compared with the SS-$13^{BN}$ rats. Importantly, unlike NO, the Ang II–stimulated O$_{2}^-$ produced in the mTAL of SS/Mcw1 rats diffused to the surrounding vasa recta pericytes. O$_{2}^-$ cross-talk from mTAL to vasa recta was not observed in control SS-$13^{BN}$ rats, as was the case in previous studies in tissue strips of normal Sprague-Dawley rats. Cross-talk of O$_{2}^-$ from mTAL to vasa recta has only been observed previously in tissues pretreated with an NO scavenger (eg, carboxy-PTIO). The SS/Mcw1 rat strain, therefore, appears to be unique in this regard and represents the first naturally occurring state in which this phenomenon has been observed.

Greater Ang II–stimulated production of O$_{2}^-$ within the mTAL of the SS/Mcw1 rats was not secondary to salt-induced hypertension, because the studies were carried out in rats maintained on a low-salt diet since weaning. Prehypertensive SS/Mcw1 rats have been found to exhibit elevated expression of reduced nicotinamideadenine dinucleotide phosphate oxidase protein in the outer medulla when compared with SS-$13^{BN}$ rats,$^8$ which may account, in part, for the greater O$_{2}^-$ production. Importantly, it was shown recently that the reduced nicotinamide-adenine dinucleotide phosphate oxidase inhibitor apocynin, when infused chronically into the renal medulla of SS/Mcw1 rats, significantly reduced the salt-induced hypertension,$^6$ again indicating that this pathway may be importantly involved in the development of hypertension in this rat strain. Taken together, it appears that the excess production and diffusion of O$_{2}^-$ in the SS/Mcw1 rat strain contributes to the reduced tissue NO levels and bioavailability that have been found previously in the outer medulla of SS/Mcw1 rats.$^{20}$

Although mTAL cells appear to be the major source of the free radicals that can diffuse and influence the contractile state of the vasa recta pericytes under normal conditions, other cell types, such as medullary interstitial cells or infiltrated inflammatory cells, could also participate in attenuating the protective effects of NO in this region. We have also examined previously whether vasa recta endothelial cells would exhibit enhanced O$_{2}^-$ production that could contribute to the damping of the pericyte NO responses by diffusing from the endothelium to the surrounding pericytes. However, it was found that Ang II did not increase pericyte O$_{2}^-$ in isolated vasa recta vessels either in the presence or absence of vasa recta endothelium.$^7$ Zhang et al$^{23}$ have similarly reported that Ang II (10$^{-8}$ mol/L) did not significantly increase the oxidation of DHE to Eth in descending vasa recta pericytes even with a 40-minute exposure to Ang II.

**Perspectives**

It is now recognized that both NO and O$_{2}^-$ can be important determinants of renal medullary blood flow, glomerular filtration, and, consequently, blood pressure.$^6,12,13,19–22,24$ It is recognized that chronic reductions of medullary blood flow can result in sustained hypertension in rats$^{9,20,21}$ and in tubular necrosis and interstitial fibrosis in the outer medulla.$^{11,25}$ Medullary blood flow is reduced in the SS/Mcw1 rats in response to a high-salt diet in contrast to Dahl salt-resistant rats$^{19}$ or Sprague–Dawley rats.$^{26}$ The present results confirm that NO and O$_{2}^-$ produced in the mTAL can diffuse to the pericytes of the surrounding vasa recta circulation and show that if production of O$_{2}^-$ is endogenously enhanced in mTAL as occurs in the SS/Mcw1 rat, NO tubulovascular cross-talk is blunted. This would be expected to increase the vulnerability of the SS/Mcw1 kidney to the vasoconstrictor and sodium-retaining actions of Ang II. Because Kobori et al$^{27}$ have found recently that intrarenal angiotensinogen was upregulated in Dahl S rats when fed a high-salt diet, the related responses of these reactive oxygen species could contribute importantly to the hypertension and renal end-organ damage, such as interstitial medullary fibrosis,$^{25}$ that develops early during salt-induced hypertension in the SS/Mcw1 rat. These novel findings in the SS/Mcw1 rat are of particular interest given the extent to which the SS/Mcw1 rat mimics the renal dysfunction found in the human salt-sensitive form of hypertension most prevalent in blacks.$^{16}$

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**Disclosures**

None.

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