Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature

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Schnackenberg, Christine G. Physiological and pathophysiological roles of oxygen radicals in the renal microvasculature. Am J Physiol Regulatory Integrative Comp Physiol 282: R335–R342, 2002; 10.1152/ajpregu.00605.2001.—The renal microvasculature is an important component in the regulation of kidney function. Recent studies suggest that oxygen radicals can contribute to the modulation of renal cortical and medullary microvascular function under normal conditions as well as in pathophysiological conditions such as diabetes mellitus and hypertension. This review focuses on studies that indicate oxygen radicals can cause renal vasoconstriction, mediate the vasoconstriction of other agonists, and modulate nitric oxide-dependent actions in the normal kidney. Hypertension and diabetes mellitus are associated with oxidative stress. Recent investigations suggest that oxygen radicals may contribute to the enhanced renal vascular tone, increased sensitivity to vasoconstrictors, impaired endothelium-dependent vasodilation, and enhanced tubuloglomerular feedback found in these pathophysiological conditions.

nitric oxide; hypertension; diabetes; kidney; vasoconstriction; superoxide; antioxidants

OXYGEN RADICALS have well-established roles in the physiology of signal transduction, cell growth, and inflammation and in the pathophysiology of cancer, aging, atherosclerosis, radiation injury, and ischemia-reperfusion injury (22). Fewer investigations have been conducted to elucidate the function of oxygen radicals in the kidney. In the past, these studies focused on reactive oxygen species in renal injury, including ischemic renal failure, transplant rejection, acute glomerulonephritis, and nephrotoxic drugs (3). There is now accumulating evidence, however, that oxygen radicals may participate in the regulation of the renal microvasculature not only during dysfunction but also under normal conditions. This review will first briefly discuss the generation, degradation, and targets of oxygen radicals. Second, the review will highlight the studies that suggest a role for oxygen radicals in the physiological regulation of the renal microvasculature. Finally, the roles of reactive oxygen species during renal microvascular dysfunction in diabetes mellitus and hypertension will be discussed.

WHAT ARE OXYGEN RADICALS?

Oxygen radicals are produced endogenously under normal conditions, and the levels are increased under conditions of oxidative stress. The most common oxygen radicals are superoxide \( (O_2^-) \), hydrogen peroxide \( (H_2O_2) \), and hydroxyl radical \( (OH^-) \) (14, 22). Whereas the anions superoxide and hydroxyl radical are more reactive, \( H_2O_2 \), which does not possess the chemical structure of a radical, is more membrane permeable. Several enzymes located throughout the cell, including in the plasma membrane, cytosol, mitochondria, and peroxisomes, generate oxygen radicals. Superoxide is produced during normal mitochondrial respiration and by NADH oxidase, NADPH oxidase, xanthine oxidase, cyclooxygenase, lipooxygenase, and cytochrome \( P-450 \). Under conditions where tetrahydrobiopterin is limited, superoxide can also be produced from nitric oxide synthase (NOS). Superoxide spontaneously gains an electron to form \( H_2O_2 \); however, three isoforms of superoxide dismutase (SOD) also catalyze this reaction. Mn-SOD is located in mitochondria, and two isoforms of Cu,Zn-SOD are located either extracellularly.
larly or intracellularly. Because native SOD is a large molecular weight molecule with limited membrane permeability, several pharmacological agents have been developed to mimic SOD including 2,2,6,6-tetramethyl-1-piperidinoxyl (TEMPO), 4-hydroxy TEMPO (TEMPOL), 2-ethyl-2,5,5-trimethyl-3-oxazolidinooxyl, and Mn(III)tetrakis(4-benzoic acid) porphyrin chloride. TEMPOL is a cyclic nitroxide that is membrane permeable, metal independent, stable, and active both in vitro and in vivo (27). Once produced, H$_2$O$_2$ can be scavenged to water by catalase or by glutathione peroxidase in the presence of reduced glutathione. Decomposition of H$_2$O$_2$ in the presence of a trace metal such as Fe$^{2+}$ produces hydroxyl radical, also known as the Fenton reaction (see Fig. 1).

In addition to the endogenous enzyme antioxidants SOD, catalase, and glutathione peroxidase, there are also scavenging antioxidants and metal binding proteins that aid in the prevention of oxidative stress. The most common scavenging antioxidants include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), carotenoids, flavonoids, uric acid, bilirubin, and thiols. Ascorbic acid is a water-soluble antioxidant and a first line of defense in plasma to inhibit lipid peroxidation. Alpha-tocopherol and beta-carotene are lipid-soluble antioxidants that act synergistically to prevent lipid peroxidation in membranes and lipoproteins. Metal binding proteins are involved in reducing hydroxyl radical formation and include hemoglobin, myoglobin, transferrin, metallothionein, ferritin, and ceruloplasmin. Another characteristic of TEMPOL is that in vitro it enhances the catalase mimic activity of metmyoglobin (MbFe$^{III}$), thus facilitating H$_2$O$_2$ dismutation (23). Overall, TEMPOL is a SOD mimetic that not only reduces the direct effects of superoxide, but also the superoxide-driven Fenton reaction that produces hydroxyl radical. In addition, TEMPOL increases H$_2$O$_2$ dismutation, but it is not a catalase mimetic per se.

The three main targets of oxygen radicals are lipids, proteins, and DNA. Extensive lipid peroxidation in biological membranes causes alteration in fluidity, decreased membrane potential, increased permeability to hydrogen and other ions, and eventual rupture of the cell. Oxidation of proteins changes their primary structure, including the overall charge, folding, and hydrophobicity. Oxidatively modified proteins are susceptible to increased aggregation and degradation. Oxygen radical-induced damage of DNA includes changes in both DNA structure and chemistry, with the result being strand breakage. Whether oxygen radicals attack these targets depends on the delicate balance between levels of reactive oxygen species vs. antioxidants. Under many conditions, an increase in oxygen radical formation signals the activation of antioxidant enzymes to aid in the increased metabolism necessary to achieve redox balance. However, when the amount of radicals produced exceeds the resources for metabolism, oxidative stress results.

**PHYSIOLOGICAL ROLES OF OXYGEN RADICALS IN THE RENAL MICROVASCULARITY**

Several investigations demonstrated various functions of reactive oxygen species in the peripheral vasculature, and these studies have been reviewed elsewhere (26, 33, 42). This review will focus exclusively on the reports of oxygen radicals in the renal cortical and medullary microcirculation. In the renal microvasculature, oxygen radicals cause vasoconstriction, mediate the vasoconstriction of other agonists, and modulate nitric oxide (NO)-dependent actions.

**Oxygen radicals are renal vasoconstrictors.** Depending on the vascular bed and oxygen radical, reactive oxygen species can cause either vasodilation or vasoconstriction (26, 33). Studies conducted thus far suggest that superoxide causes vasoconstriction in the renal cortical and medullary microcirculation. In isolated, perfused renal afferent arterioles, paraquat-induced superoxide production causes vasoconstriction that is inhibited by the SOD mimetic TEMPOL (37).

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**Fig. 1.** Production and degradation of oxygen radicals. TEMPOL, 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinoxyl; SOD, superoxide dismutase; GSH, reduced glutathione; GSSG, oxidized glutathione.

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*mitochondrial respiration*  
*NAD(P)H oxidase*  
*xanthine oxidase*  
*cyclooxygenase*  
*lipoxygenase*  
*cytochrome P450*  
*nitric oxide synthase*  

 TEMPOL  

**Mn-SOD, Cu,Zn-SOD**  

*O$_2^-$  

H$_2$O$_2$  

Fe$^{3+}$  

Catalase  

GSH  

H$_2$O + GSSG  

Glutathione peroxidase  

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However, TEMPOL alone had no affect on afferent arteriolar tone, suggesting that oxygen radicals do not participate in maintaining basal tone of in vitro microperfused rabbit afferent arterioles. Furthermore, microperfusion of TEMPOL into the efferent arteriole of Wistar-Kyoto (WKY) rats did not alter the stop-flow pressure at tubular perfusions of 0 or 40 nl/min (44), again indicating that oxygen radicals do not affect basal cortical microvascular tone. In contrast, TEMPOL infusion into the renal medullary interstitium markedly increases medullary blood flow in the anesthetized rat, and infusion of an inhibitor of SOD decreased medullary blood flow (46). In addition, Rhinehart and Pallone (32) recently showed that TEMPOL dilates preconstricted rat outer medullary descending vasa recta. These studies suggest that, unlike in the cortical microvasculature, superoxide does participate in maintaining basal tone of the renal medullary microcirculation.

This regional difference may be due to the greater capability of the renal outer medulla to produce oxygen radicals. Because the medulla has a lower Po2 than the cortex, oxygen radical formation under basal conditions appears to be increased in the deeper region of the kidney (46). The higher basal levels of oxygen radicals may participate in the regulation of renal medullary blood flow and water and electrolyte excretion under physiological conditions. Indeed, Zou et al. (46) demonstrated that in association with the selective decrease in medullary blood flow (no change in cortical blood flow) during interstitial infusion of an inhibitor of SOD, urine flow and sodium excretion decreased in anesthetized rats. In contrast, TEMPOL infusion under the same conditions increased medullary blood flow, urine flow, and sodium excretion.

There are several potential sources of superoxide in the renal microcirculation. Immunocytochemistry, RT-PCR, or Western analysis has identified all subunits of the NADPH oxidase enzyme in the rat kidney (5, 13). Fluorescence spectrometry of renal tissue suggests that superoxide is produced by NADH oxidase > NADPH oxidase ≈ mitochondrial respiration > xanthine oxidase in the cortex and by NADH oxidase > mitochondrial respiration > NADPH oxidase > xanthine oxidase in the outer medulla (46). Although there are several possible sites for oxygen radical generation in the cortical and medullary microvasculature, NADPH oxidase appears to be one of the major sources.

Oxygen radical-induced renal vasoconstriction could be mediated by both direct and indirect means. Superoxide can increase intracellular calcium concentrations in vascular smooth muscle cells and in endothelial cells through several different mechanisms (26). 8-Isoprostane PGF<sub>2α</sub>, which is the product of oxygen radical's nonenzymic attack on arachidonic acid, causes preferential vasoconstriction of the preglomerular vasculature principally through activation of a thromboxane A<sub>2</sub> (TXA<sub>2</sub>) receptor (41). In addition, superoxide may inhibit the release of a vasodilator or stimulate the production of a vasoconstrictor. For example, superoxide inhibits prostacyclin synthase activity, thereby blocking the production of PGI<sub>2</sub> (48). Recently, Chen et al. (6) showed that superoxide increases the production and renal vasoconstrictor actions of adenosine. Whether some or all of these mechanisms play a role in oxygen radical-induced vasoconstriction of the renal microcirculation remains to be determined.

**Oxygen radicals participate in stimulated renal vasoconstriction.** Because oxygen radicals are extracellular signaling molecules, they may be important in mediating the actions of other renal vasoconstricting agents. ANG II, TXA<sub>2</sub>, endothelin-1 (ET-1), and norepinephrine are powerful vasoconstrictors of the renal microvasculature. Superoxide has been shown to be permissive in the TXA<sub>2</sub>-induced vasoconstriction of in vitro microperfused afferent arterioles (37). Scavenging of superoxide with TEMPOL completely prevents the vasoconstriction induced by TXA<sub>2</sub> receptor activation with U-46,619 (10<sup>−10</sup>−10<sup>−6</sup> M) or by ANG II (10<sup>−10</sup>−10<sup>−6</sup> M). In contrast, TEMPOL only partially blocks afferent arteriolar vasoconstriction induced by ET-1 and norepinephrine. The renal afferent arteriolar vasoconstrictor responses to higher doses of ET-1 (10<sup>−9</sup> M) and norepinephrine (10<sup>−6</sup> M) are not blocked by scavenging of superoxide (author’s unpublished observations). Although the physiological role of oxygen radicals as signaling molecules for vasoconstriction in the normal kidney needs to be further elucidated, studies in the peripheral vasculature in normal animals and humans have shown similar effects. In the rat mesenteric microcirculation (20) and aorta (19) and in the human forearm (9), ANG II-induced vasoconstriction is significantly attenuated after treatment with SOD or vitamin C. On the other hand, norepinephrine-induced vasoconstriction in the aorta, similar to that in the renal afferent arteriole, is not significantly altered by SOD (19).

Whether or not oxygen radicals mediate renal vasoconstriction caused by other agents may be dependent on their selective activation of enzymes that produce superoxide. Studies by Sorescu et al. (39) suggest that NADPH oxidase is the major source of superoxide in vascular smooth muscle (VSM) cell membranes; whereas mitochondrial respiration, xanthine oxidase, arachidonate-derived enzymes, and NOS are relatively minor sources. ANG II-induced superoxide formation in VSM cells appears to be mediated by activation of the AT<sub>1</sub> receptor and subsequent upregulation of the Nox1 (a novel gp91 phox) subunit of NADPH oxidase (24). ANG II-induced superoxide production in mesangial cells is also mediated through activation of AT<sub>1</sub> receptors and NADPH oxidase and protein kinase C (17). Furthermore, ET-1 induces NADPH oxidase in human vascular endothelial cells (10). Whether physiological concentrations of renal vasoconstrictors such as ANG II, TXA<sub>2</sub>, ET-1, or norepinephrine activate NADPH oxidase or other radical-generating enzymes in the renal microvasculature remains to be determined.

**Superoxide restricts NO-dependent action.** Several studies suggest that the oxygen radical superoxide interacts with NO and thus limits its bioavailability.
The affinity of NO for superoxide is so high that its rate of reaction is limited only by diffusion. Because superoxide effectively degrades NO to peroxynitrite, the biological activity of NO may be determined by the availability of superoxide. This superoxide-mediated quenching of NO-dependent action appears to have a physiological role in the renal microcirculation.

NO modulates the renal vasoconstriction caused by agonists such as ANG II, TXA2, and ET-1. Many studies indicate that these agents stimulate NO production, which then acts to buffer the vasoconstriction. However, it is unclear why this agonist-induced NO production does not override the vasoconstriction and result in a vasodilation. Studies using in vitro microperfused rabbit afferent arterioles suggest that superoxide limits the amount of NO-mediated buffering of TXA2-induced vasoconstriction (37). The vasoconstrictor response of afferent arterioles to U-46,619 is turned into a vasodilator response in the same vessels pretreated with TEMPOL. This vasodilatory response to U-46,619 during TEMPOL is blocked in the vessels pretreated with the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). These data suggest that superoxide plays an important role in limiting the amount of NO buffering TXA2 receptor-stimulated vasoconstriction in renal afferent arterioles. Because studies in the peripheral vasculature have shown that ANG II and ET-1 can stimulate superoxide and NO, it seems possible that their interaction may be playing a role in the renal microvasculature response to ANG II and ET-1 also.

In addition, recent reports indicate that superoxide limits the buffering capability of NO in tubuloglomerular feedback (TGF). Microperfusion of the NO donor S-nitroso-N-acetyl-penicillamine (SNAP) into the lumen of the macula densa produces graded buffering of TGF, and microperfusion of TEMPOL into the efferent arteriole blunts the maximal TGF response of WKY rats (45). This study further demonstrated that the acute vasodilator responses to SNAP are enhanced after simultaneous microperfusion of TEMPOL and suggests that superoxide and NO interact in the juxtaglomerular apparatus to modulate the TGF response under normal conditions. Whether oxygen radicals participate in the overall regulation of glomerular filtration rate through the modulation of TGF remains to be determined. Because oxygen radicals can alter TGF through stimulation of adenosine production (6) and through altering NO signaling at the macula densa, more studies are needed to address whether oxygen radicals contribute to the complex response to long-term alterations in salt intake. Because chronic changes in salt diet alter the activity of the renin-angiotensin system, NO system, and (directly or indirectly) the production of oxygen radicals, the overall renal hemodynamic response to changes in salt intake will be an integration of these components and remains to be determined.

The role of superoxide in restricting NO-mediated vasodilation in the renal medulla is less clear. Using the NO fluorescent indicator 4,5-diaminofluorescein, Rhinehart and Pallone (32) demonstrated that stimulation of NO with bradykinin is significantly enhanced by TEMPOL in isolated rat outer medullary descending vasa recta. However, Zou et al. (46) showed that the increase in renal medullary blood flow and sodium excretion during renal medullary interstitial infusion of TEMPOL was only partially blocked by L-NAME in anesthetized rats. The difference in these results highlights the need to further integrate isolated vessel experiments with in vivo studies to understand fully the role of oxygen radicals and antioxidants in the medullary microvasculature. In addition, studies demonstrate that NO modulates ANG II-induced vasoconstriction in the renal medulla (47). Whether superoxide restricts NO-mediated buffering of ANG II-induced vasoconstriction of the medullary vasculature still remains to be determined.

Although studies investigating the roles of oxygen radicals in the physiological regulation of the renal microcirculation have only recently begun, it is evident that oxygen radicals have important direct and indirect actions in both the cortical and medullary microcirculation. Oxygen radicals directly constrict the renal microcirculation and indirectly affect renal vascular tone by mediating the effects of other vasoconstrictors, stimulating the production of vasoconstrictors, and modulating the actions of vasodilators such as NO. In addition, acute studies have shown that the tonic production of oxygen radicals in the medulla causes vasoconstriction, antidiuresis, and antinatriuresis; and thereby it may contribute to the control of medullary blood flow and overall fluid and electrolyte balance. Basal production of oxygen radicals in the cortex contributes to the TGF component in the control of glomerular filtration rate. It is clear that long-term studies are now needed to discern the important roles of oxygen radicals in the overall physiological regulation of renal hemodynamic and excretory function.

PATHOPHYSIOLOGICAL ROLES OF OXYGEN RADICALS IN THE RENAL MICROVASCULATURE

Renal dysfunction is a central cause of hypertension and a common consequence of diabetes mellitus. These pathophysiological conditions set up a vicious cycle of repeated renal injury and are the two leading causes of end-stage renal failure in the United States. Oxidative stress is associated with both diabetes and hypertension in humans and in experimental animal models. Therefore, this section will focus on how oxygen radicals may play an important role in the pathophysiology of the renal microvasculature in diabetes mellitus and hypertension.

Oxygen radicals in diabetes mellitus. Endothelial dysfunction in peripheral and renal vessels is a common sequela of diabetes mellitus. Although not all reports agree, some observations indicate that the tonic influence of NO in the renal microvasculature is suppressed and contributes to the endothelial dysfunction in the early stages of insulin-dependent diabetes (2). Because superoxide rapidly scavenges NO, one
possible explanation for the lack of NO influence under basal conditions in the diabetic renal microvasculature is excessive superoxide. Indeed, renal cortical tissue from diabetic rats has increased superoxide production (16). Ohishi and Carmines (30) demonstrated that the afferent and efferent arteriolar vasoconstrictor response to the NOS inhibitor N-nitro-L-arginine (L-NNA) is impaired in juxtamedullary nephrons of streptozotocin-diabetic rats. Treatment with SOD restored the vasoconstrictor response to L-NNA. Similarly, vasodilatory responses of isolated renal arteries to SOD in streptozotocin-diabetic rats were greater than in control rats (8). These studies indicate that increased superoxide reduces the modulation by NO of basal tone in renal microvessels in diabetes.

In addition to an impaired basal NO influence, the stimulation of NO-dependent vasodilation by a number of agonists is also impaired in diabetic kidneys and may be due to elevations in oxygen radicals. In in vitro microperfused afferent arterioles from insulin-dependent diabetic rabbits at 10 days, acetylcholine-induced vasodilation was impaired and restored toward control after acute treatment with TEMPO (34). However, SOD treatment did not improve acetylcholine-induced vasodilation in renal arteries isolated from diabetic rats at 6 wk (8). The conflicting results may be due to the differences in the duration of diabetes, the vessels studied, or membrane permeability of the enzyme antioxidant.

Since superoxide limits the buffering capability of NO during agonist-induced vasoconstriction in the renal cortical microcirculation under normal conditions, it seems possible that under conditions of oxidative stress, such as in diabetes mellitus, that the buffering capability of NO during agonist-induced vasoconstriction is decreased. Indeed, Schoonmaker et al. (38) showed that the renal afferent arteriolar responsiveness to ANG II is enhanced in juxtamedullary nephrons from diabetic rats and that L-NNA did not alter the response. However, treatment with SOD restored the ability of L-NNA to enhance the vascular response to ANG II (1). These data suggest that excess superoxide is responsible for the lack of NO buffering of ANG II-induced vasoconstriction of afferent arterioles in diabetes.

The increased oxygen radicals in the diabetic renal microvasculature may be due to selective and time-dependent changes in antioxidant activities in the kidney. Changes in antioxidant enzyme activities were comprehensively evaluated in a longitudinal study of kidneys isolated from rats 0–6 wk after induction of diabetes (18). The study demonstrated that total and Cu,Zn-SOD activities were increased 1–6 wk after diabetes, but that Mn-SOD activity was not different from controls. Renal glutathione peroxidase activity is also increased during 1–6 wk of diabetes, but there is a biphasic response of renal catalase activity to diabetes. In the kidney, catalase activity was increased 1 wk after diabetes, returned to control, and was then decreased at 5–6 wk. The activity of scavenging antioxidants was also variable in diabetic kidneys. Craven et al. (7) reported that vitamin E but not vitamin C was decreased in renal cortex of rats after 2 mo of diabetes. However, there are conflicting reports of the antioxidant activities in glomeruli isolated from diabetic rats (16, 31, 40). Because several antioxidants exist in multiple cell types of the kidney, further studies are needed to identify which antioxidant activities are altered specifically in the renal microvasculature. Overall, what may be lacking in the diabetic renal microvasculature is the normal compensatory upregulation of key antioxidant enzymes and scavengers in the presence of an increase in oxygen radical production.

The sources of increased oxygen radical formation in diabetes are similar to those found under normal conditions in the kidney but with the additional factor of glucose. As previously mentioned, ANG II stimulates superoxide formation through activation of NADPH oxidase, which is increased in the retina of the BBZ/Wor diabetic rat (12). In streptozotocin-diabetic rats, angiotensin-converting enzyme inhibition attenuates the oxidative stress in the kidney and decreases albuminuria (21). Together, these studies intimate that ANG II-induced oxygen radical formation may play a role in the oxidative stress of the diabetic renal microvasculature. Increasing evidence suggests, however, that hyperglycemia is a main source of oxygen radical formation in diabetes. Elevated plasma glucose concentrations can increase oxygen radical production through glucose autoxidation, the formation of advanced glycosylation end products, and metabolic stress. Increased glucose in the media of normal in vitro microperfused rabbit afferent arterioles significantly increases their sensitivity to ANG II and prevents L-NAME-induced potentiation of the vasoconstrictor response (1). Although several more studies are needed, these reports suggest that hyperglycemia and ANG II may be important sources of oxidative stress in the diabetic renal microvasculature.

**Oxygen radicals in hypertension.** Oxidative stress in the vasculature has been associated with human essential hypertension and several hypertensive animal models, including the spontaneously hypertensive rat (SHR), spontaneously hypertensive stroke-prone rat, ANG II-induced hypertension, renovascular hypertension, Dahl salt-sensitive hypertension, lead-induced hypertension, cyclosporine-induced hypertension, pre-eclampsia, obesity-induced hypertension, and DOCA-salt hypertension (42). However, because norepinephrine-induced hypertension does not alter vascular production of oxygen radicals (25), high blood pressure per se does not appear to be associated with oxidative stress in the vasculature. Most studies indicate that antioxidant treatment lowers blood pressure and improves endothelial function in large conduit vessels. The few studies that have investigated oxygen radicals in the kidney in hypertension also suggest that increased oxygen radicals are important in the renal microvascular dysfunction in hypertension.

The SHR has increased blood pressure and renal vascular resistance and an enhanced TGF response. Acute and longer-term studies suggest that oxygen radicals may play an important role in these charac-
teristics. The SOD mimetic TEMPOL normalizes the blood pressure, renal vascular resistance, and renal excretion of 8-iso-PGF₂α in SHR (35, 36). Furthermore, TEMPOL increases the basal luminal diameter of in vitro perfused afferent arterioles of juxtamedullary nephrons in SHR while having no effect in WKY (15). These studies suggest that oxygen radicals may contribute to the increased blood pressure and renal microvasculature resistance in the SHR.

One of the potential mechanisms for the acute reduction in blood pressure and renal vascular resistance in SHR treated with antioxidants is via enhancing NO action in the renal vasculature or in the juxtaglomerular apparatus. Systemic inhibition of NO synthesis with L-NAME blocks the acute antihypertensive actions of TEMPOL in SHR (36). This study implies that NO-mediated vasodilation may be restored after scavenging of oxygen radicals in the SHR, but the renal vascular response was not determined. However, Ichihara et al. (15) demonstrated that afferent arteriolar vasoconstrictor responses to neuronal (n) NOS inhibition with S-methyl-L-thiocitrulline or nonselective inhibition of NOS with L-NNA are enhanced in arterioles pretreated with TEMPOL in SHR but not in WKY. Investigators also show that while nNOS inhibition with 7-nitroindazole (7-NI) treatment enhances TGF in WKY, the response is not altered in SHR (44). Microperfusion of TEMPOL restores the enhancing effect of 7-NI on TGF in SHR to the level observed in WKY.

In more recent studies, Welch and Wilcox (43) showed that 2-wk treatment with candesartan in SHR restored a normal TGF response to 7-NI in SHR compared with WKY. Intermedullary infusion of TEMPOL increases the basal luminal diameter of in vitro perfused afferent arterioles of juxtamedullary nephrons in SHR while having no affect in WKY (15). These studies suggest that oxygen radicals may contribute to the increased blood pressure and renal microvasculature resistance in the SHR.

The actions of oxygen radicals in the renal microcirculation in hypertension may not be limited to the cortex. Dukacz et al. (11) demonstrated that the renal medullary blood flow response to ANG II is enhanced in SHR compared with WKY. Intermedullary infusion of L-arginine abolished the enhanced response to ANG II in SHR, and L-NAME infusion enhanced the response in WKY. Ten-week treatment with the angiotensin-converting enzyme inhibitor enalapril decreased the sensitivity of the renal medullary circulation to ANG II in SHR. Because previous studies showed that ANG II can stimulate oxygen radicals in the vasculature, it is possible that the lack of NO buffering in the medullary circulation of the SHR may be due to increased ANG II-induced oxygen radical formation.

ANG II-induced stimulation of oxygen radical formation, which can diminish NO action, is not specific to the SHR. Nishiyama et al. (29) demonstrated that the increased blood pressure and renal vascular resistance in ANG II-infused hypertensive rats were ameliorated by TEMPOL. NOS inhibition markedly attenuated the hemodynamic response to TEMPOL. These studies strongly implicate ANG II as a major source of oxygen radical formation in ANG II-dependent or ANG II-sensitive forms of hypertension.

Another possible mechanism for the renal protective actions of antioxidants in hypertension is through a reduction in the immune and inflammatory responses. Reactive oxygen species can act as signal transduction messengers for several transcription factors including nuclear factor (NF)-κB, which plays a critical role in the activation of multiple genes that contribute to the inflammatory response and end organ damage. ANG II-dependent hypertension (28) and DOCA-salt hypertension (4) animal models have increased NF-κB activation in the kidney and renal monocyte/macrophage infiltration. In association with a reduction in vascular oxygen radical formation, TEMPOL also reduces the blood pressure and NF-κB activation and monocyte/macrophage infiltration in the kidneys of DOCA-salt hypertensive rats (4). In double (human renin and angiotensinogen-)transgenic rats (28), a reduction in renal NF-κB activation and monocyte/macrophage infiltration also decreases blood pressure, renal vascular injury, and albuminuria. Therefore, some of the renal vascular

![Fig. 2. Regulation of renal microvascular tone: role for oxygen radicals. VSM, vascular smooth muscle; GFR, glomerular filtration rate; TGF, tubuloglu-
merular feedback; NO, nitric oxide; ET, endothelin; HETEs, hydroxytetraenoic acids; EETs, epoxyciso-
atrienolic acids.](http://ajpregu.physiology.org/ by 10.220.33.1 on July 5, 2017)
damage in hypertension may be due to the proinflammatory actions of oxygen radicals in the kidney.

SUMMARY

There are several factors that contribute to the regulation of renal microvascular tone (see Fig. 2). These include paracrine and autocrine factors, such as ANG II, adenosine, hydroxytetraenoic acids, and epoxyeicosatrienoic acids, which act on VSM cells to cause vasoconstriction or vasodilation. The endothelium produces satrienoic acids, which act on VSM cells to cause vaso-

II, adenosine, hydroxytetraenoic acids, and epoxyeico-

include paracrine and autocrine factors, such as ANG


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