Oxidative stress and nitric oxide deficiency in the kidney: a critical link to hypertension?

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Oxidative stress implies an increased production, or a decreased scavenging or metabolism, of reactive oxygen species (ROS). Pioneering work by Harrison, Griendling, and colleagues (53, 162) established that ANG II-induced hypertension in the rat is accompanied by oxidative stress in blood vessels. They showed further that administration of forms of superoxide dismutase (SOD) that interact with endothelial cells reduce blood pressure (BP) in this model but not in rats with norepinephrine-induced hypertension, which do not develop oxidative stress (102). They implicated increased activity of reduced NADP (NADPH) oxidase in the generation of oxidative stress in the blood vessel wall. Subsequent studies by Schiffrin and Touyz (76, 155, 161, 235), Harrison (193), Webb (11), Manning (122), Schmid-Schobein (201) and colleagues showed further that oxidative stress is engendered during mineralocorticoid (MC)-salt hypertension and salt-loaded Dahl salt-sensitive or spontaneously hypertensive stroke-prone rats (SHRsp), in which the renin-angiotensin-aldosterone system (RAAS) is suppressed, thereby widening the role of oxidative stress in hypertension. Indeed, an increase in salt intake without accompanying hypertension also can enhance renal NADPH oxidase, impair SOD, and cause oxidative stress (91).

Many authors have shown that antioxidants can diminish not only the increase in BP but also the inflammation, fibrosis, sclerosis, and dysfunction of the heart, kidneys, and other organs of certain hypertensive models (10, 50, 59, 67, 68, 90, 92, 93, 100, 126, 158, 177, 195, 215–217, 258–261, 268). However, in one study of oxidative stress, caused by prolonged infusion of endothelin-1 (ET-1) into rats, the hypertension was resistant to an effective antioxidant regimen with Tempol (43). This conflicts with a prior study in which Tempol administered...
to ET-1 infused rats abolished the increase in blood pressure and renal vascular resistance, while it corrected the abnormal lipid peroxidation (186). The cellular and organ-sparing effects of antioxidants in hypertensive models appear to be at least partially independent of any BP lowering (25, 92, 256, 265). Studies of human subjects with essential or renovascular hypertension report evidence of oxidative stress (97) that underlies the endothelial dysfunction of forearm blood vessels observed in vivo (54, 154) or of isolated vessels studied ex vivo (66, 240). Inhibition of nitric oxide synthase (NOS) causes hypertension and renal and systemic vasoconstriction in normal human subjects, which demonstrates the importance of ongoing nitric oxide (NO) generation for the maintenance of normal BP and blood flow (21, 72, 224). The evidence of the primary role of the kidneys in setting the long-term level of BP (32, 64) has focused attention on renal mechanisms of hypertension mediated through oxidative stress and NO deficiency. This is the subject of the present review, which places special emphasis on studies from the author’s laboratory that formed the basis for the Starling lecture.

Among ROS, attention has centered on the free radical, highly reactive superoxide anion ($O_2^-$) and the more stable hydrogen peroxide ($H_2O_2$). Reaction of $H_2O_2$ with metals, notably Fe$^{2+}$, leads directly to the formation of the highly reactive hydroxyl radical (•OH). •OH is also generated by a reaction between $O_2^-$ and NO (74). The reactions of $O_2^-$ or •OH with NO not only lead to NO bioinactivation (63, 94), and thereby to endothelial dysfunction (173), but to the generation of highly oxidative and nitrosating species, including peroxynitrite (ONOO$^-$) (221, 234). Hypochlorous acid (HOCI$^-$) is generated by myeloperoxidase in activated phagocytes and can circulate to cause widespread endothelial dysfunction (42). These species represent only some of the proximate ROS. Their further reaction with cell products can lead to long-lasting mediators. These include oxidized low-density lipoprotein (LDL) formed from the interaction of H$^+$ or •OH with LDL, or isoprostanes formed by the interaction of $O_2^-$ with arachidonic acid, or advanced glycation end products formed by the interaction of ROS with carbohydrate moieties, or carbonyl species formed by the interaction of ROS with proteins, or oxidized DNA and its products, or nitrosated tyrosine epitopes on proteins. Because presently less is known of the importance of these “second-order” oxidation products in the hypertensive kidney, precedence is given to $O_2^-$ and $H_2O_2$.

This review aims to summarize evidence linking ROS to hypertension, with an emphasis on renal and vascular mechanisms.

CAN ROS CAUSE HYPERTENSION?

Four lines of evidence indicate that ROS can cause hypertension. First, interventions designed to cause oxidative stress lead to the gradual development of hypertension. Vaziri and colleagues have documented two examples. Lead promotes •OH and $O_2^-$ generation and lipid peroxidation in cultured aortic and vascular smooth muscle cells (39, 138). Rats given lead in their drinking water develop oxidative stress and nitrotyrosine deposition in their blood vessels and organs (230) and hypertension (62), despite upregulation of endothelial nitric oxide synthase (eNOS) (228, 229). The effect appears to be a direct action of lead to induce ROS generation since there are no major changes in the expression of NADPH oxidase, glutathione peroxidase, SOD, or catalase (231). The hypertension depends on ROS, as it can be prevented by coadministration of a free radical scavenger, vitamin E (230) or of an OH$^-$ scavenger, dimethylthiourea (40). In a second model, rats given buthionine sulfoximine for 2 wk in the drinking water to deplete glutathione likewise develop oxidative stress, nitrotyrosine deposition, and hypertension (233, 282). Again, the hypertension apparently depends on ROS, as it is prevented by coadministering vitamins E and C (233).

Second are observations that oxidative stress can precede hypertension. Studies in 4-wk-old SHR show enhanced renal expression of the p47phox component of NADPH oxidase and increased plasma levels of lipid peroxidation products, whereas BP does not rise until after this age (25).

Third are studies of gene-deleted mice. Deletion of extracellular superoxide dismutase (EC-SOD) leads to oxidative stress and increased basal blood pressure whether measured while conscious with blood pressure telemetry or under anesthesia (31, 251). Gene transfer of EC-SOD into SHR reduces their BP (31). Furthermore, deletion of the gene for fibulin-5, which is essential for binding of EC-SOD to vascular tissue (137), also raises the BP of mice (135, 273). Because EC-SOD metabolizes $O_2^-$ to $H_2O_2$, these findings relate hypertension specifically to an increase in $O_2^-$, or a decrease in $H_2O_2$, in the extracellular environment. EC-SOD activity depends on a supply of Ca$^{2+}$ by the Ca$^{2+}$-binding protein Atox-1. Remarkably, fibroblasts from Atox-1 -/- mice not only have defective function of EC-SOD but also have a greatly reduced EC-SOD expression, whereas both Atox-1 and EC-SOD are upregulated in blood vessels of mice with atherosclerosis (84). Clearly, Atox-1 and Fibulin-5 are important regulators of vascular EC-SOD, but their effects in the kidney have not been studied.

Fourth are many reports that the correction of the oxidative stress that accompanies a variety of hypertensive models also corrects hypertension (10, 50, 59, 67, 68, 90, 92, 93, 126, 158, 177, 195, 215–217, 258–261, 268).

RENAL GENERATION OF ROS DURING HYPERTENSION

Evidence of increased ROS in the hypertensive kidney comes from three primary sources. First are observations of increased steady-state renal excretion of lipid peroxidation markers in hypertensive animals. This provides quantitative evidence of increased ROS, but increased renal excretion is not in itself evidence of renal ROS production. 8-Isoprostane PGF$_{2\alpha}$ and malondialdehyde (MDA) excretion are increased by prolonged infusion of ANG II into rats (24, 248) or mice (89) and in ANG II-dependent forms of hypertension, including the spontaneously hypertensive rat (SHR) (256) and the two-kidney one-clip (2K,1C) Goldblatt model of renovascular hypertension (249). Equally impressive increases are found in volume-dependent models, including Dahl salt-sensitive (122, 124, 125, 210) and DOCA-salt (11) rats. The SHR and ANG II-infused rabbit have increased plasma levels of lipid peroxidation products (LPOs) (25, 238).

Second are observations of increased renal tissue generation of ROS or ROS-dependent products. There is increased renal nitrotyrosine immunoreactivity in kidneys of SHR (253), 2K,1C rats (13), those with aortic banding (9), lead-induced
oxidative stress and hypertension (230), and chronic renal insufficiency (232). Interestingly, nitrotyrosine immunoreactivity in the 2K,1C model is located preferentially in the postclip kidney around cells of the macula densa, whereas in the contralateral kidney, it is located preferentially within the walls of the afferent arteriole (13). These are sites of elevated ANG II since the postclip kidney generates excessive renin within the juxtaglomerular apparatus (JGA), whereas the contralateral kidney is perfused with blood with high renin-angiotensin content. Because nitrotyrosine implies ROS interaction with NO, these findings indicate that ANG II-induced O$_2^*$ formation is increased in the JGA of the postclip kidney and the afferent arteriole of the contralateral kidney. ROS in the JGA could be quenched by reaction with NO generated by neuronal nitric oxide synthase (nNOS) expressed in macula densa cells (266), and ROS in the afferent arteriole could be quenched by NO generated by eNOS expressed in the afferent arteriolar endothelial cells (213).

Third are more direct measurements of ROS. Zou and Cowley (284) have shown O$_2^*$ production in the medulla of rats. Tojo and colleagues (145) demonstrated increased renal production of H$_2$O$_2$ by an ANG type 1 receptor (AT$_1$R)-dependent mechanism in rats with insulinopenic diabetes mellitus.

***RENA L EXPRESSION OF NOS, OXIDASES, AND ANTIOXIDANTS IN HYPERTENSION***

Endothelial or type III NOS is expressed abundantly in renal vascular endothelium (213), in the thick ascending limb (TAL) of Henle’s loop (151) and in the collecting ducts (196). Neuronal NOS (nNOS) type I is expressed abundantly in the macula densa cells (133, 266), Bowman’s capsule and the inner medullary collecting ducts (211, 214). The mRNA and protein for both nNOS and eNOS are increased in the JGA of the SHR (252) (Fig. 1). This is accompanied by a two-fold increase in renal cortical nitrotyrosine deposition, indicating enhanced interaction of NO with ROS (253). Dietary salt restriction in normal rats increases the expression of nNOS in the kidney cortex and macula densa (14, 167, 184, 191, 212, 213) but not in the collecting ducts (191). This suggests differential regulation of NO by salt in the renal cortex and medulla. Indeed, dietary salt restriction diminishes expression of all nOS isoforms in the renal inner medulla (123). Dietary salt restriction enhances the expression of eNOS in the kidney cortex and vascular endothelium (212). The effects of salt on renal cortical nNOS expression are independent of ANG II, since they are unaffected by AT$_1$-receptor blockade during salt restriction or by ANG II infusion during salt loading, whereas similar maneuvers ascribe the effects of salt on the expression of eNOS to ANG II acting on AT$_1$ receptors (212).

NADPH oxidase in activated neutrophils is composed of a membrane-associated heterodimer of the flavoprotein gp91phox with p22phox that is activated by binding of a cytoplasmic component, p47phox, which is itself activated by p67phox and rac-1. The role of p40phox is uncertain (5). NADPH oxidase activity can be reconstituted in vitro using the purified cytosolic factors p47phox, p67phox, and Rac with phospholipid-reconstituted gp91phox and p22phox (49). However, activity can be achieved in the absence of p47phox if there is a high concentration of p67phox and Rac (57). Moreover, homologous components can substitute of gp91phox (nox-1 or -4) (2), and homologs to p47phox and p67phox (p41nnox and p51nnox) can support O$_2^*$ production by NADPH oxidase in some cells (55). The components of neutrophil NADPH oxidase are all present in endothelial cells. However, in these cells, the majority of gp91phox, p22phox, p67phox, and p40phox and of NADPH ox-
dase activity is located on the intracellular cytoskeleton in a
perinuclear region, whereas p47phox is expressed predomi-
nantly in cell membranes (108). The functions of these prin-
cipal NADPH oxidase components have been evaluated thor-
oughly in the activated phagocyte, where gp91phox, p22phox,
and p47phox, p67phox and rac-1 are required to support maximal
rates of $O_2^-$ generation. Mutations in these subunits in patients
with chronic granulomatous disease lead to defective phago-
cyte killing of bacteria and recurrent infection. However, the
requirement of these components for $O_2^-$ generation at other
sites is incompletely understood and is a subject of intense
investigation.

Chabrashvili et al. (25) detected the mRNA and protein for
gp91phox, p22phox, p47phox, and p67phox in the rat renal cortex.
Immunohistochemical studies located prominent sites of ex-
pression of these proteins in the arterioles [vascular smooth
muscle cells (VSMCs) and endothelium], the glomerulus, and
the distal nephron. Remarkably, all four proteins are expressed
strongly at the luminal border of the macula densa cells. Podocyte
cells in the glomerulus are prominent sites for p47phox expression.
The SHR kidney has increased expression of these components,
notably of p47phox protein and mRNA. The increased p47phox is
detected in the kidney, coincident with increased plasma LPO in
4-wk-old SHR before the establishment of hypertension.

Fig. 2. Means ± SE values for basal, xanthine-, NADH-, and NADPH-stimulated $O_2^-$ generation in homogenates of renal cortex (A) or renal outer medulla (B) of rabbits
infused for 2 wk with a sham infusion or with ANG II at a subpressor rate of 60 ng·kg$^{-1}$·min$^{-1}$ (60) or at a slow pressor rate of 200 ng·kg$^{-1}$·min$^{-1}$ (200). Compared
with sham: *P < 0.05; **P < 0.01. ns, nonsignificant. [From Wang et al. (238).]
O$_2^-$ and/or H$_2$O$_2$ also can be generated in the kidney and blood vessels by xanthine oxidoreductase (X-OR) (69, 200). When assessed by lucigenin-detected O$_2^-$ in response to specific substrates and enzyme antagonists, X-OR activity is negligible in homogenates from the rabbit renal cortex, but in the medulla, is equivalent to NADPH oxidase (238). However, X-OR appears to be regulated differently from NADPH oxidase. Thus NADPH oxidase activity in the kidney cortex (238, 248), but not in the outer medulla, of the rabbit or rat is upregulated by ANG II (238), but X-OR activity is unchanged at either site (238) (Fig. 2).

However, when assessed from fluorogenic oxidation of ethidium, NADH oxidase is of predominant importance in both the cortex and outer medulla of the rat kidney, although there is almost no oxidase activity in the papilla (284). This difference between the studies may relate to the availability of cofactors that determine the interconversion of NADH and NADPH oxidase activity. O$_2^-$ also can be generated by lipoxygenase (78–80, 98, 187, 286), epoxygenase (46), and NOS (98, 286). Uncoupling of eNOS has been detected in blood vessels of hypertensive rats, where oxidation of tetrahydrobiopterin (BH$_4$) to dihydrobiopterin (BH$_2$) by ROS produced by NADPH oxidase (98) or by ONOO$^-$ (286) directs NOS to synthase O$_2^-\cdot$ rather than NO. If a similar reaction occurs in the kidney, this would provide a ready mechanism, whereby generation of O$_2^-$, for example by activation of NADPH oxidase, could further enhance ROS by oxidizing BH$_4$ and uncoupling NOS (98).

There are several examples of discordant signaling by ROS. Although O$_2^-$ or ONOO$^-$ can oxidize BH$_4$, which can lead to further O$_2^-$ generation from NOS, H$_2$O$_2$ can increase the synthesis of BH$_4$ in cultured endothelial cells by transcriptional upregulation of guanosine triphosphate-cytohydroxylase I, which is the limiting step in BH$_4$ synthesis (187). O$_2^-\cdot$ biodegrades NO, whereas H$_2$O$_2$ enhances eNOS expression and NO activity in cultured endothelial cells sufficiently to increase NO release (20). This can explain how the addition of ANG II to cultured endothelial cells paradoxically increases their NO activity, despite oxidative stress. This increase in NO is prevented by catalase, which metabolizes H$_2$O$_2$ and is absent from cells of p47$^{phox}$ knockout mice (20). Thus the interaction between ROS and NO in vascular tissue is complex and involves both direct effects and compensatory mechanisms. Despite these reservations, direct measurement of O$_2^-$ and NO in blood vessels using electron spin paramagnetic resonance demonstrates that 7 days of ANG II infusion into rats doubles the vascular activity of O$_2^-$ but halves the vascular activity of NO (127). Apparently, bioactivation of NO by O$_2^-\cdot$ is of predominant importance in determining NO activity in blood vessels during prolonged exposure to ANG II in vivo (Fig. 3).

SOD is expressed as three isoforms: mitochondrial (Mn), intracellular (IC), and extracellular (EC) (50). All are expressed in the normal kidney (24, 248). SOD catalyzes the conversion of O$_2^-\cdot$ to H$_2$O$_2$, thereby changing a highly reactive radical that inactivates NO (87) into a more stable compound whose functions include the release of the endothelium-derived hyperpolarizing factor (EDHF) (71, 112, 175), upregulation of eNOS (20), and hypertrophic and remodeling responses in blood vessels (50). These two ROS have discordant effects in the renal medulla on the generation of hypertension, as BP is increased by medullary H$_2$O$_2$, rather than O$_2^-\cdot$, both in acute and in more prolonged studies (30, 120, 121). However, the dominant effect of EC- and IC-SOD activity normally is to metabolize O$_2^-\cdot$ and thereby prevent hypertension. Thus blockade of glutathione production, which should increase intracellular H$_2$O$_2$, causes progressive hypertension (231, 233). EC-SOD knockout mice have a higher BP during the early phase of high-dose ANG II infusion that can be normalized by acute intravenous injection of EC-SOD (87). SOD mimetic nitroxides, such as Tempol, whether given acutely or during pro-

Fig. 3. Fold changes in rats infused with ANG II for 7 days, compared with controls, for aortic mRNA expression of NADPH oxidase components, Nox-1 and p22$^{phox}$ (A), protein expression of eNOS (B), activity of protein kinase C (C), and O$_2^-\cdot$ generated from NADPH and vascular NO bioavailability assessed from electron paramagnetic resonance (D). Significance of change from control: *P < 0.05. [From Mollnau et al. (127).]
longed administration, lower the BP in multiple models of hypertension (176, 178). Therefore, interest is focused on overexpression of NADPH oxidase components, or underexpression of SOD, as potential prohypertensive mechanisms in models of ANG II, MC, or salt-induced hypertension. Indeed vascular EC-SOD is upregulated by NO and by exercise, which could be a component of the antihypertensive effects of these maneuvers (51).

Blood vessels from rats or rabbits infused with ANG II generate increased O$_2^-$ and have increased expression of p22$^{phox}$, p67$^{phox}$, and a Nox homolog (24, 53, 56, 99, 127, 218, 237, 238) (Fig. 4). During ANG II infusion, one homolog of gp91$^{phox}$, Nox-1, is upregulated in VSMCs (73, 99, 127, 199) and the kidney (24). Another homolog, Nox-4, is upregulated in blood vessels of ANG II-infused rats (127) or in VSMCs from renin-transgenic rats (267) but is downregulated by ANG II in cultured VSMCs from normal rats (99). Studies of normal rats infused with ANG II alone, or with an AT$_1$- or AT$_2$-receptor antagonist, indicate that renal cortical mRNA expression for Nox-4 is downregulated strongly by ANG II. This is ascribed to an inhibitory action of ANG II on Nox-4 expression mediated via AT$_1$-R (24). gp91$^{phox}$ is upregulated by ANG II in blood vessels and endothelial cells (86, 127) and in VSMCs of human resistance arteries (218), but little gp91$^{phox}$ is expressed in the media of human coronary blood vessels (194). Coronary arteries from patients with atherosclerosis have prominent expression of gp91$^{phox}$ in macrophages but prominent expression of Nox-1 in VSMCs (194).

ANG II infusion upregulates the expression of EC-SOD in rat blood vessels (50, 52) yet downregulates its expression in the rat kidney (24, 248).

Infusion of ANG II at a slow pressor rate increases NADPH oxidase activity in the renal cortex of the rat (24, 248, 281) and rabbit (238) without significant upregulation of NADH oxidase or X-OR (Fig. 2). There is increased expression of p22$^{phox}$ and Nox-1 and downregulation of Nox-4 (24) (Fig. 4). Renal afferent arterioles of rabbits infused with ANG II have enhanced expression of the mRNA for p22$^{phox}$ (237, 238). These effects of ANG II in the kidney are mediated by AT$_1$-R, as they are prevented by candesartan (24). The upregulation of p22$^{phox}$ and Nox-1 is counteracted by stimulation of AT$_2$-R, which also downregulates the expression of p67$^{phox}$ (24). ANG II infusion in the rat decreases the renal cortical mRNA for EC-SOD yet increases Mn-SOD (24). The decrease in EC-SOD is due to activation of AT$_1$-R (Fig. 4). NADPH oxidase likely is of predominant importance for O$_2^-$ generation in the renal cortex since more O$_2^-$ is generated with NADPH than with NADH or xanthine as substrates (238, 284). In contrast, NADPH and XO contribute equally to O$_2^-$-generating capacity in the outer medulla of the rabbits (238, 284). This demonstrates important regional differences in the ANG II-
dependent regulation of ROS and NOS, and hence the bioactivity of NO, in the kidney. Nevertheless, despite the absence of a notable increase in oxidative stress in the outer medulla or in isolated, perfused vasa recta of ANG II-infused rats, in situ studies demonstrate that ANG II infusion increases $O_2^\bullet$- generation in medullary TAL segments. The $O_2^\bullet$- generated at this site interacts with NO in vasa recta to mediate vasoconstriction and enhance tubular Na$^+$ reabsorption. When given into the medulla, Tempol must be coadministered with catalase to cause vasodilation or to reduce BP in some studies (30, 120), although in others Tempol alone is effective (284). This is in contrast to the vasodilatation and antihypertensive effects of systemic Tempol, or Tempol applied directly to isolated blood vessels, where catalase is not required to elicite these effects.

A high salt intake increases the NADPH oxidase activity of the rat kidney cortex and increases the excretion of 8-isoprostane PGF$_{2\alpha}$ and MDA, despite profound reductions in the circulating RAAS. Opposite changes occur during a low-salt intake (91). High salt increases the renal cortical expression of mRNA for gp91$^{phox}$ and p47$^{phox}$ and decreases IC- and mitochondrial-SOD. Thus both salt loading and prolonged ANG II can generate oxidative stress and engage the renal machinery for $O_2^\bullet$- generation via NADPH oxidase while impairing $O_2^\bullet$- metabolism via SOD, but by discrete changes in the components of these systems (Fig. 4). This may explain how infusion of ANG II during a high-salt intake may cause severe oxidative stress, hypertension, and renal damage (22). Remarkably, salt loading and ANG II regulate the same endpoint in these complex systems, but by engaging different components.

Early insulinopenic diabetes mellitus in the rat, which, like salt loading, is a model of low renin and volume excess, enhances the renal expression of gp91$^{phox}$ and p47$^{phox}$ (4). In this model, preventing membrane assembly of p47$^{phox}$ with apocynin prevents an increase in renal and glomerular H$_2$O$_2$ generation and excretion of lipid peroxidation products (4).

**DOPAMINERGIC ACTIONS ON ROS**

At high concentrations, dopamine and D$_1$-receptor-like agonists act as prooxidants that increase $O_2^\bullet$- and H$_2$O$_2$ (12, 26, 60, 117, 241, 257), ROS are produced both by dopamine transporter uptake-dependent and dopamine receptor-independent mechanisms (23, 115). Excessive stimulation of D$_2$ receptors increases ROS production (269). D$_3$ receptors stimulate phospholipase D activity which can activate NADPH oxidase (44).

At physiological concentrations, dopamine acts as an antioxidant. Activation of D$_3$ receptors by apomorphine protects cells against the lethal effects of oxidative stress via generation of cycle guanosine 5'-monophosphate (cGMP)-operated Ca$^{2+}$ channels (81). Dopamine, acting at the D$_1$ and D$_3$ receptor, decreases oxidative stress in brain cortical cells (139) and renal tubular and vascular smooth muscle cells (274, 277) by inhibition of phospholipase D activity (277) and stimulation of SOD and catalase activities (134). The D$_3$ receptor, which is one of the two members of the mammalian D$_1$-like receptor family, has antioxidant properties in renal proximal tubules and brain mediated by inhibition of phospholipase D$_2$ and NADPH oxidase activity (274). D$_5$ receptor−/− mice are hypertensive and have increased oxidative stress in the kidney and brain (75, 274). Apocynin, a drug that inhibits the assembly of NADPH oxidase subunits, normalizes blood pressure and oxidative stress in the kidney and brain of D$_2$ receptor−/− mice (275).

An additional antioxidant mechanism of the D$_3$ receptor may derive from its ability to decrease the expression of the AT$_1$ receptor in renal proximal tubules (279). Thus at physiological concentrations, dopamine protects against hypertension, in part, by activation of D$_3$ receptors that prevent oxidative stress in the kidney.

**SELF-SUSTAINING NATURE OF OXIDATIVE STRESS**

Several mechanisms have been implicated in sustaining oxidative stress. Stretching blood vessels increases $O_2^\bullet$- generation, thereby providing a direct link between hypertension and ROS production in conduit vessels (222). ANG II infusion increases renal cortical expression of the mRNA and protein for p22$^{phox}$, yet prevention of oxidative stress with Tempol also prevents upregulation of p22$^{phox}$ and NADPH oxidase activity in the kidney (248). This indicates that oxidative stress may enhance expression of p22$^{phox}$ and NADPH oxidase activity itself. Indeed, generation of ROS or addition of H$_2$O$_2$ to endothelial cells in culture increases p38 mitogen-activated protein kinase and phosphatidylinositol-3-kinase and enhances expression of p22$^{phox}$ mRNA and protein (41). ANG II infusion downregulates EC-SOD in the kidney, which should enhance levels of $O_2^\bullet$- (24). Oxidative stress can oxidize BH$_4$ sufficiently to uncouple eNOS and thereby direct NOS to generate $O_2^\bullet$- rather than NO in blood vessel walls (103, 286). ONOO$^-$ generated during oxidative stress can nitrosate and inactivate IC-SOD (1). Indeed, vascular SOD activity in stroke-prone SHR is increased, and NADPH oxidase activity is decreased, after prolonged treatment with vitamins C and E (27). H$_2$O$_2$ stabilizes TP receptors in the membrane, prevents recycling, and, thereby, increases the number of apparent binding sites for TP receptor agonists (223). ANG II infusion reduces prostaglandins and thromboxane A$_2$ (TxA$_2$) (116). Studies in the thromboxane-prostanoid-receptor (TP-R) knockout mouse show that this receptor mediates oxidative stress during a slow pressor infusion of ANG II (88). Thus ANG II-induced H$_2$O$_2$ generation can perpetuate vasoconstriction and oxidative stress mediated via the TP. Antioxidants reduce the influx of inflammatory cells into the kidneys of SHR, thereby reducing a major source for further ROS generation (169). These are some of the pathways whereby oxidative stress could become self-sustaining (Fig. 5).

**ROS AND RENAL FUNCTION**

The role of ROS in the regulation of renal function has been studied from the consequences of enhancing ROS metabolism or inactivation. Intravenous injections (178) or prolonged administration (176) of the permanent SOD mimetic nitroxide, Tempol into SHR reduce BP and renal vascular resistance (RVR). Normotensive Wistar Kyoto (WKY) rats have a blunted acute response and no fall in BP with prolonged Tempol administration (176, 250). Blockade of NOS prevents most of the fall of BP during chronic Tempol infusion in the SHR (178), yet less than half of the response of a bolus dose of Tempol in control WKY (270), SHR (156), or in DOCA-salt.
rats (271, 271, 272). Thus, unlike chronic treatment, only a part of the acute response to Tempol can be attributed to enhanced bioactivity of NO by a reduction in tissue $\text{O}_2^\cdot$ concentration. Indeed, the acute fall in MAP is accompanied by bradycardia (178, 271, 272), rather than the tachycardia that is provoked by an infusion of an NO donor compound such as sodium nitroprusside (SNP) (7). These NO-independent effects of acute Tempol have been attributed to reductions in $\text{O}_2^\cdot$ in peripheral sympathetic nerves that inhibit their activity (270, 272). Direct application to peripheral nerves of the SOD inhibitor diethyl-dithiocarbamate increases their activity, whereas direct application of Tempol has the opposite effect (188).

The reductions in RVR of SHR infused with Tempol are in proportion to the falls in MAP, thereby maintaining renal blood flow. Similarly, long-term studies in which Tempol is given to SHR (176) or to 2K,1C renovascular hypertensive rats (249), or to ANG II-infused mice (89) or rats (248) show that Tempol is highly effective in moderating the increase in BP, yet reduces RVR only in proportion to the reduction in MAP. These studies in rats did not detect significant changes in the glomerular filtration rate (GFR) with Tempol, although Tempol does increase the GFR of ANG II-infused mice (89). Majid, Nishiyama, and colleagues (118, 119) studied anesthetized ANG II-infused dogs. They confirmed that Tempol reduces RVR acutely and leads to a diuresis and natriuresis. They showed further that the renal vasoconstriction and especially the natriuresis and diuresis with Tempol persist after NOS blockade. They concluded that the tubular effects of systemic Tempol are independent of NO.

Kawada et al. (89) reported that coadministration of Tempol to mice infused with a low dose of ANG II over 2 wk prevented the increase in renal excretion of 8-isoprostane PGF$_2\alpha$, the slow development of hypertension, and the increase in RVR. The reduction in RVR was attributed to preferential inhibition of pregglomerular vasoconstriction, since Tempol increased GFR in this mouse model yet maintained the ANG II-induced increase in filtration fraction. The preferential vasoconstriction of afferent arterioles by $\text{O}_2^\cdot$ in the mouse could represent a direct effect on the renal afferent arteriole or an indirect effect mediated through enhancement of the tubuloglomerular feedback (TGF) response (255, 260, 263).

H$_2$O$_2$ has complex effects on the tone of blood vessels. Preliminary studies in mice have disclosed a biphasic effect of H$_2$O$_2$ on mesenteric and cremasteric vessels in vivo (29). Superfusion of mesenteric blood vessels with H$_2$O$_2$ yields vasodilation in TP-R$^+_m$ mice. This may reflect release of an EDHF, as shown in human submucosal intestinal vessels challenged with Ach (71). The vasoconstriction seen in wild-type mice precedes the vasodilation and may reflect stabilization of TP-Rs in the membrane by H$_2$O$_2$, and, hence, facilitation of contractions mediated via the TP-R (223). H$_2$O$_2$ reduces the diameter of isolated, perfused renal afferent arterioles from the rabbit (179). These studies have shown that ROS can have both vasoconstrictor or vasodilator actions depending on conditions. SOD has an important regulatory role in converting $\text{O}_2^\cdot$, which is predominantly a vasoconstrictor, to H$_2$O$_2$, which has bifunctional vasodilator/vasoconstrictor properties.

Johnson and colleagues have shown that a 2-wk high dose ANG II infusion in the rat is followed in the succeeding weeks by salt sensitivity (113). Thus the BP of these rats is normal but rises progressively during a high-salt intake. The ANG II infusion leads to a persistent inflammatory cell infiltrate in the

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Fig. 5. Some proposed mechanisms of oxidative stress that could be self-sustaining in blood vessels or the kidneys. For explanation, see text.
renal cortical interstitium that is linked both to the development of salt sensitivity and to oxidative stress in this model. Moreover, salt sensitivity, afferent arteriolar vasoconstriction and remodeling, and oxidative stress are prevented in these rats by mycophenolate mofetil that prevents the inflammatory infiltrate (47, 168). Thus ANG II may induce oxidative stress in the kidney either by upregulation of NADPH oxidase components resident in tubular, glomerular, and vascular cells or by causing a persistent influx of phagocytes that can generate substantial ROS. The interstitial influx of oxidase-bearing cells is particularly important in the oxidative stress that accompanies severe hypertension and renal damage induced by high levels of ANG II (114), whereas ROS generated by resident renal cells is likely predominant in less severe forms of hypertension.

There is a delayed increase in BP during ANG II infusion into p47phox knockout mice (97), whereas there is an exaggerated early increase in BP during ANG II infusion into EC-SOD knockout mice (87). This demonstrates the importance of NADPH oxidase-derived \( \cdot O_2^- \) in the development of hypertension during ANG II infusion. TP-R knockout mice have a blunted rise in BP and no increase in RVR with ANG II (48, 88). Moreover, these mice fail to develop oxidative stress with ANG II infusion (88). Apparently, ANG II activates TP receptors to engage oxidative stress, which can account for about one half of the increase in BP and all of the increase in RVR. The increase in ROS may entail TP-R-dependent proinflammatory actions that enhance cellular immune responses (203). Additionally, TP-Rs can activate protein kinase C (PKC) in VSMCs and kidney glomeruli (33, 65) that upregulates NADPH oxidase and enhances ROS formation in resident renal and vascular cells (143, 244).

**AFFERENT ARTERIOLE IN HYPERTENSION**

Because structural and functional adaptation of the renal afferent arteriole can contribute to the development of hypertension and may be a focal point for prohypertensive actions of ROS in the kidneys, this review will include an overview of mechanisms of oxidative stress in blood vessels with specific reference to the afferent arteriole in hypertension.

Autopsy studies of patients with hypertension by Moritz and Oldt (130) reveal that the renal afferent arteriole is a preferential site for structural adaptation. The afferent arteriole shows narrowing of the lumen, hypertrophy of the wall, and hyalinization or myointimal proliferation in 98% of those known to have had hypertension during life, compared with only 12% of normotensive subjects. Blood vessels to other organs show a lesser frequency of structured remodeling in hypertension. Tracy and colleagues (219, 220) have perfusion-fixed kidneys and measured the mean afferent arteriolar diameter. They have documented a continuous relationship between reduced luminal diameter of cortical afferent arterioles and the premortem level of BP. Mulvany and colleagues (141) have shown that the lumen of the afferent arteriole is narrowed in hypertensive SHR and hypertensive primates (192). Among F1 hybrid crosses between SHR and WKY, the development of hypertension in adult life is predicted by a reduced afferent arteriolar diameter in the prehypertensive state (141).

The glomerular capillary hydraulic pressure \( (P_{GC}) \) and blood flow to outer cortical glomeruli of SHR are well maintained despite the presence of hypertension (82). This implies an increased pleglomerular vascular resistance. Studies have confirmed excellent autoregulation of outer cortical blood flow in the SHR during short-term step changes in perfusion pressure (82). However, there is less complete autoregulation of blood flow to juxtamedullary nephrons, which have an elevated \( P_{GC} \) and an accelerated rate of glomerulosclerosis (82) and associated tubular damage (142). The extent to which this pleglomerular vasoconstriction of SHR outer cortical glomeruli is an autoregulation to sustained hypertension or a primary defect that engenders hypertension is not clearly resolved. Guyton’s model of circulatory homeostasis predicts that an increase in afferent arteriolar resistance or TGF responsiveness will displace the BP: body salt relationship to the right, leading to sustained, salt-resistant hypertension (64). This is consistent with the predominantly salt-resistant hypertension of the SHR (61), which has a narrowed afferent arteriole (141) and an enhanced TGF response (252). An enhanced distal NaCl reabsorption is required to add a component of salt sensitivity (32, 64). Moreover, because dietary salt loading can itself increase ROS generation in the kidney and blood vessels (91, 122) and ROS are linked to hypertension, it remains quite possible that oxidative stress could contribute to renal vascular mechanisms of both salt-resistant and salt-sensitive hypertension. Therefore, an enhanced reactivity or remodeling of afferent arterioles under the influence of ROS could underlie hypertension during renal oxidative stress. The importance of renal afferent arteriolar remodeling has been emphasized by Johnson and colleagues (85).

The ANG II slow pressor response is a gradual rise in BP over days or weeks during an infusion of ANG II at an initially subpressor rate. This response is seen in mice (89), rats (16, 105, 106, 116, 146, 147, 248), rabbits (38, 237, 238), and humans (3). It is relatively specific for ANG II, as infusions of norepinephrine lead to tachyphylaxis (3, 102), although a similar slow pressor response develops in rats infused with a TP-R mimetic, U-46,619 (245). The hypertension is preceded by a selective increase in RVR (77, 89). The responses in the rabbit, rat, and mouse are accompanied by increased ROS and increased excretion, or increased renal or vascular production, of lipid peroxidation products (24, 52, 53, 68, 88, 89, 97, 102, 146, 147, 162, 237, 238, 243, 248, 256, 261, 278) and increased nitrotyrosine deposition implying ONOO− generation (93). The hypertension and renal vasoconstriction depend on \( O_2^- \) because they are prevented by coinfusion of a membrane-permeable form of SOD (102) or by the nitroxide SOD mimetic, Tempol (89, 146, 147, 248).

Further studies implicated vasoconstrictor PGs, including TxA2, whose excretion is increased during an ANG II slow pressor response (116). Coffman and colleagues (48) and Kawada and colleagues (88) have reported that the ANG II pressor response is blunted in mice with targeted disruption of the TP-R (182). Indeed TP-R−/− mice have a paradoxical reduction in RVR with ANG II and fail to develop oxidative stress. Thus TP-Rs mediate oxidative stress, hypertension, and renal vasoconstriction of afferent arterioles during an ANG II slow pressor response.

**ROS AND TGF**

TGF entails a predominant increase in pleglomerular (afferent arteriolar) resistance, leading to a fall in \( P_{GC} \) and single
nephron glomerular filtration rate (SNGFR) during delivery and reabsorption of solute at the macula densa segment of the nephron (15). TGF contributes to renal autoregulation (160), and thereby guards glomerular capillaries against barotrauma during hypertension. Resetting of TGF permits the timely and efficient excretion of NaCl in response to changes in BP or salt intake and thereby guards against salt sensitivity and hypertension (18, 128, 144, 180). Wilcox et al. (266) and Mundel et al. (133) reported heavy expression of nNOS in the macula densa cells of the rat. Microperfusion of the NOS inhibitor Nω-nitroarginine (L-NMA) or of an NO scavenging molecule (pyocyanin) into the tubular lumen of the macula densa or into the blood supplying the interstitium of the JGA via the efferent arteriole enhances the TGF response (266). Microperfusion of L-NMA reduces PGC during a TGF response but is ineffective if the TGF response is quiescent during luminal furosemide or in the absence of luminal salt delivery (266). These studies establish that NO is generated in the macula densa during luminal NaCl reabsorption and blunts the TGF response. Use of the relatively nNOS-specific inhibitor 7 nitroindazole (7-NI) confirms that it is this isoform that participates in TGF responses in the rat (252, 253, 255). Studies of nNOS blockade show that nNOS is responsible for the resetting of TGF and glomerular hemodynamics after sustained changes in salt intake or proximal tubule reabsorption (18, 204, 205, 208). Moreover, blockade of nNOS in the normal rat reduces the GFR and leads to hypertension (144).

Studies of pharmacologic blockade of nNOS in the macula densa of the rat or of the nNOS knockout mouse (225) or of the isolated, doubly-perfused JGA preparation from the rabbit (151) have shown that nNOS blunts the reduction in PGC or SNGFR during macula densa reabsorption of solute. There is normally a lower SNGFR when assessed from tubular fluid samples drawn from the early distal tubule (DT), compared with the proximal tubule (PT), since PT sampling disrupts flow to the macula densa and thereby interrupts the contribution of macula densa activation and TGF to the reduction of SNGFR. Accordingly, the finding by Vallon, Schnermann and colleagues that this difference in SNGFR between PT and DT is enhanced in nNOS−/− mice implies that nNOS in the macula densa tonically enhances SNGFR via the macula densa (225). However, it is currently unclear why this group detected no differences in TGF responses to tubular fluid perfusion of the macula densa segment of nNOS−/− mice (225).

The finding that macula densa cells express both nNOS (266) and a complete complement of NADPH oxidase components (25) provoked the hypothesis that TGF responses are regulated by an interaction between O₂⁻ and NO (253, 260). Despite a twofold upregulation of the protein and mRNA for nNOS in the renal cortex and immunocytochemical confirmation that this upregulation involves the macula densa cells (252) (Fig. 1), the SHR has diminished or absent responses of PGC to microperfusion of 7-NI into the macula densa, implying no role for nNOS-derived NO in blunting of TGF responses (253). This cannot be ascribed to a failure to deliver adequate substrate (L-arginine) or a critical cofactor (BH₄), as luminal microperfusion of these substances fails to restore a normal response to nNOS inhibition in SHR nephrons. The NO donor compound SNP was perfused into the lumen of the MD to investigate the bioactivity of luminally delivered NO in the JGA (253, 256). SNP caused graded increase in PGC, consistent with blunting of TGF. The response to SNP was more sensitive, but not more responsive, in SHR nephrons. The response to SNP in SHR nephrons was enhanced and became similar to the WKY, during contemporary microperfusion of Tempol into the efferent arteriole supplying the test nephron.

The implication from these studies is that endogenous NO produced in the macula densa of the SHR is bioactivated by O₂⁻ in the JGA before exercising its influence to dampen TGF. This was examined directly from the increase in TGF responses during blockade of macula densa nNOS by luminal microperfusion of 7-NI (256) (Fig. 6). Microperfusion of 7-NI enhances TGF responses of WKY, but not of SHR, nephrons. Contemporary microperfusion of Tempol into the efferent arteriole does not modify the enhancement of TGF responses to luminal 7-NI in the WKY, yet results in a six-fold enhancement of the response to luminal 7-NI in the SHR. Parallel studies were undertaken in WKY and SHR that were administered the AT₁ receptor antagonist, candesartan, or triple antihypertensive therapy with hydralazine, hydrochlorothiazide, and reserpine (HHR), which does not reduce renin or block AT₁ receptors (256). Both treatments yielded equivalent reductions in BP in SHR to the levels of normal WKY. Neither treatment altered the TGF responses of WKY. In contrast, prolonged candesartan administration to SHR normalized the enhancement of TGF by luminal 7-NI, and completely prevented the augmented responses to luminal 7-NI in the SHR nephrons during efferent arteriolar microperfusion of Tempol. Whereas HHR had no significant effects, there was a tendency for a reduced TGF, which could relate to antioxidant effects of hydralazine. Apparently, TGF responses are enhanced in SHR nephrons because of a failure to blunt TGF by nNOS-derived NO. This can be ascribed to a local interaction of NO with O₂⁻ within the JGA that leads to bioactivation of NO. The enhanced generation of O₂⁻ in the JGA of the SHR can be attributed to AT₁-receptors. It occurs independent of changes in systemic BP, or local expression of nNOS in the macula densa (255, 256, 258, 260, 262, 265).

The interaction between O₂⁻ and NO has been studied by Garvin and colleagues (164, 165) in the isolated, doubly perfused JGA and TAL segments (148–152, 159, 197, 226) of rabbit nephrons. NO inhibits the absorption of NaCl in the TAL by the generation of cGMP that inactivates the luminal Na⁺/H⁺ exchanger (152). Higher concentrations of NO also inhibit the basolateral Na⁺/K⁺-ATPase (226). eNOS is the principal source of NO in the TAL (159), whereas nNOS is the principal source in the macula densa (258) and collecting ducts (197). O₂⁻ enhances NaCl reabsorption in the TAL, in part, by inactivation of the effects of locally generated NO to prevent luminal Na⁺ entry (149, 150) or to block Na⁺/K⁺-ATPase (226). Thus one mechanism whereby O₂⁻ may increase afferent arteriolar tone is bioactivation of NO within the JGA. This should augment TGF responses by enhancing the TGF signal generated within the macula densa cells during luminal solute delivery and absorption (164).

TGF responses depend on ANG II (181). Thus TGF is enhanced by infusion of ANG II (181), is blunted during administration of an angiotensin converting enzyme inhibitor or an angiotensin receptor blocker (ARB) to rats (198, 254) and is absent in the AT₁A receptor knockout mouse (183). ANG II activates AT₁ receptors that are expressed on macula densa cells (70), where it increases intracellular calcium concentra-
tion (ICCa2/H11001) (83, 111) that can activate NOS and generate NO (206, 207). Likewise, ANG II activates AT_1 receptors expressed on the renal afferent arteriole (238), where it also increases ICCa2/H11001 (239, 283), releases NO (157, 206, 207), and generates O2/H11002/H18528 (238). AT1- and TP-R, PKC-dependent activation of NADPH oxidase (33, 65, 127, 143, 244) could provide a mechanism for generation of ROS in macula densa cells and renal afferent arterioles during infusion of ANG II that offsets the effects of NO (237, 260).

REDOX REGULATION OF THE RENAL CORtical AFFERENT ARTERIOLE AND MEDULLARY VASA RECTA

Studies of isolated, pressurized, and perfused renal afferent arterioles from mice (157) and rabbits (237, 238) provide direct insight into the role of ROS in enhanced microvascular reactivity during prolonged ANG II infusion. These vessels from normal animals have a robust relaxation response to Ach that entails an endothelium-dependent relaxation factor (EDRF) and an EDHF. The EDRF depends on NOS, while the EDHF response is mediated by an epoxyeicosatrienoic acid (EET) derivative that activates a potassium conductance (236).

ROS are implicated in the short-term (minutes) and longer-term (days) responses of renal afferent arterioles to several vasoconstrictors. Incubation of rabbit afferent arterioles for 20 min with the TP-R mimetic U-46,619 induces dose-dependent contractions that are powerfully modulated by local generation of NO and ROS. Thus contractions are enhanced by blockade of NOS but dampened by metabolism of O2/H11002/H18528 by Tempol (179).
Afferent arterioles dissected from the kidneys of rabbits infused with ANG II at a slow pressor rate exhibit an increased expression of mRNA for p22phox and COX-2, but a maintained expression of COX-1 and TP-R (264), and a reduced expression of AT1-R (237, 238). No transcripts for AT2-R are detected. Therefore, functional studies investigated the roles of O2· derived from NADPH- and PGs derived from COX-2. Contractions to ANG II are increased two-fold in arterioles from rabbits infused with ANG II despite downregulation of the AT1-R. This is accompanied by enhanced responsiveness to ET-1 and U-46,619 but persistent responses to norepinephrine (NE) and high [K+] in arterioles from normal rabbits (237). This maintained response to two vasococontractors indicates that the enhanced ANG II response in this model of early adaptation to infused ANG II is probably not secondary to structural remodeling. Removal of the endothelium of arterioles from normal rabbits enhances contractions to ANG II, thereby demonstrating that ANG II releases an EDRF from normal arterioles. In contrast, removal of the endothelium from arterioles of ANG II-infused rabbits blunts contractions to ANG II, thereby demonstrating that ANG II releases an endothelial-derived contracting factor (EDCF) from arterioles of ANG II-hypertensive rabbits (237).

The nature of the EDCF was studied from response to Ach in arterioles from ANG II-infused rabbits without preconstriction (Fig. 7). Vessels were incubated with L-nitro-arginine to block EDRF/NO and 14,15 epoxyeicosa-5(Z)-enoi acid (14–15 EEZE) to block EET-induced EDHF (236). Vessels from sham-infused rabbits do not contract to Ach, whereas those from ANG II-infused rabbits show a graded contraction (Fig. 7). This contraction is abolished by endothelium removal and therefore is an EDCF response. The EDCF is moderated, but not abolished, by bath addition of Tempol (SOD mimetic) to metabolize O2· or by SC-506 (COX-1 antagonist) or by OKY-046 (thromboxane A2 synthase antagonist) (TxA2-S). However, the contractions are almost abolished by bath addition of parecoxib to block COX-2 or by ifetroban to block TP-Rs (237).

These studies have disclosed two related processes that underlie enhanced contractility of afferent arterioles during an ANG II slow pressor response (Fig. 8). The first is an increased responsiveness of the VSMCs to a number of agonists including ANG II, ET-1, and TP-R mimetics that is dependent upon oxidative stress. The second is the absence of an EDRF response, due to bioinactivation of endothelial NO by O2·, and the generation of an EDCF response that depends on COX-2 generation of a TP-R ligand. The nature of this ligand is presently unclear but TxA2 is not primary, as the EDCF response is only blunted by a TxA2-S antagonist. Isoprostanes are formed nonenzymatically by interaction of O2· with arachidonate (131) and can activate TP receptors on renal arterioles (202). However, isoprostanes presumably are not the primary TP ligand either since the EDCF response is dependent on cyclooxygenase and is not abolished by metabolism of O2· with Tempol. Likely candidates include the stable prostaglandin endoperoxide, PGH2 (110) or a COX metabolite of HETE (242), perhaps oxidatively metabolized to a polar form (170).

In contrast to the enhanced reactivity of the renal cortical afferent arteriole, there is diminished reactivity to ANG II of isolated vasa recta of ANG II-infused rats (153, 166, 281, 285). The addition of ANG II to the bath of isolated vasa recta generates a large excess of NO as shown by the NO-sensitive dye, 3-amino-4-aminomethyl-2'7'-difluorofluorescin diacetate DA (281). Studies with dihydroethidium loading to detect O2· provide no evidence of oxidative stress in the vasa recta of ANG II-infused rats (281). Unlike the renal outer cortex (281), there is no increase in NADPH activity in the renal outer medulla of ANG II-infused rats (281) and no increase in NAPDH, NADH- or xanthine-oxidase in the outer medulla of rabbits undergoing an ANG II slow pressor response (238).

A limitation of some studies of the renal medulla is that they are not always performed under the unusual conditions of low Po2 encountered in the medulla in vivo. Moreover, it is possible that effects of ANG II to enhance O2· generation via the activation of AT1-Rs are concealed by coincident activation of AT2-Rs. Indeed, an increase in ROS occurs in isolated vasa recta when ANG II is added to the bath in the presence of the AT2-R antagonist PD-123,319 (280).

Fig. 7. Means ± SE values for acetylcholine-induced contractions of isolated, pressurized, perfused afferent arterioles under spontaneous tone from rabbits infused with ANG II for 12 days. Endo-, endothelium removal. Drugs added to bath: vehicle, Tempol [superoxide dismutase (SOD) mimetic], SC-506 [(cyclooxygenase) COX-1 antagonist], parecoxib (COX-2 antagonist), ifetroban [thromboxane-prostanoid receptor (TP-R) antagonist], and OKY-046 [thromboxane synthase (TxA2-S) antagonist]. Compared with vehicle: **P < 0.01; ***P < 0.005. [From Wang et al. (237).]
Studies of intact rats have shown that inhibition of SOD in the renal medulla by diethylthiocarbamate causes oxidative stress, reduces medullary blood flow, and raises BP (121, 284). Likewise, local medullary infusion of H$_2$O$_2$ (120) raises the BP. Studies of rat renal outer medullary strips with fluorescent probes for O$_2^-$/H$_2$O$_2$/NO show clearly that the addition of ANG II increases O$_2^-$/H$_2$O$_2$/NO generation in the medullary TAL segment, but not in pericytes of vasa recta (129). However, an increase in O$_2^-$ in vasa recta can derive from the adjacent TAL segments. These studies have shown how NADPH-oxidase-derived O$_2^-$/H$_2$O$_2$/NO in the TAL, if not quenched by NO, can lead to oxidative stress in the adjacent vasa recta. Collectively, the evidence shows a role for O$_2^-$ in the renal cortex and medulla of ANG II-infused rats or rabbits to eclipse the effects of NOS-derived NO on renal afferent arterioles or to influence the tone of vasa recta in the renal medulla.

DEFECTS IN RENAL OXYGENATION DURING HYPERTENSION AND OXIDATIVE STRESS

ROS ultimately derive from molecular oxygen either as a byproduct of mitochondrial respiration where O$_2^-$ generation increases with P$_{O_2}$, or as the product of specific oxidases notably NADPH, NADH oxidase or xanthine oxidase, or by interaction with various cellular constituents such as Fe$^{2+}$, whereby ·OH is generated from H$_2$O$_2$ in the Fenton reaction. Therefore, the availability of O$_2$ may regulate ROS generation, according to the functional $K_m$ of the specific system involved. Recent studies have examined the P$_{O_2}$ in the kidneys in vivo and its effects on ROS generation to address the hypothesis that P$_{O_2}$ could limit oxidative stress in the kidney.

Renal oxygen usage (Q$_{O_2}$) normally increases linearly with tubular sodium transport (T$_{Na}$) above a basal level (35, 209).
The slope of this line (T\textsubscript{Na}:Q\textsubscript{O2}) defines the efficiency with which the kidney uses O\textsubscript{2} for chemical work, above a basal level. This efficiency depends markedly on NO and ROS (104).

Hintze and colleagues reported that NOS blockade in the dog increases the Q\textsubscript{O2}, while reducing the GFR, and hence the T\textsubscript{Na}. Consequently, the T\textsubscript{Na}:Q\textsubscript{O2} declines sharply (104). Similar results are apparent in other tissues, such as the heart, where NOS blockade also enhances O\textsubscript{2} usage at a given level of work (109). Although the molecular mechanisms responsible are unclear, NO can compete with O\textsubscript{2} for mitochondrial respiration (17, 163, 174). Therefore, a reduction in NO may enhance mitochondrial O\textsubscript{2} usage above the level required to satisfy the energy needs of the cell. Because the cell has a strictly limited capacity to store unused sources of chemical energy, this will lead to an inefficient utilization of O\textsubscript{2} for chemical work.

The T\textsubscript{Na}:Q\textsubscript{O2} of the SHR kidney is reduced by 50%. As in normal kidneys after the NOS blockade (104), this is due to an enhanced O\textsubscript{2} usage despite a reduced T\textsubscript{Na} (246). This inefficient O\textsubscript{2} utilization in the SHR kidney can be corrected fully by 2 wk of administration of an ARB, whereas equiantihypertensive therapy with hydralazine, hydrochlorothiazide and reserpine is not effective (247). These results assign the inefficient O\textsubscript{2} utilization in the SHR kidney to prolonged AT\textsubscript{1} receptor activation and disassociate it from the accompanying hypertension. The early phase of 2K,1C Goldblatt hypertension in the rat is also characterized by ANG II-dependent hypertension and oxidative stress. The postclipped kidney also has a reduced T\textsubscript{Na}:Q\textsubscript{O2}. However, in the 2K,1C model, the defect in O\textsubscript{2} usage is not reversed fully by prolonged administration of an ARB but is normalized by correcting oxidative stress with Tempol (249).

The effects of ANG II infusion for 12 days at a slow pressor rate when given alone, or with the SOD mimetic Tempol, have been studied in the rat (248). ANG II increases BP, RVR, p22\textsuperscript{phox} expression, and NADPH oxidase activity of the renal cortex, and decreases the T\textsubscript{Na}:Q\textsubscript{O2} and PO\textsubscript{2} in the kidney (Fig. 9). Tempol alone has no effect, but when given with ANG II, Tempol prevents all of these changes. Remarkably, p22\textsuperscript{phox} expression is reduced below baseline in ANG II-infused rats given Tempol (Fig. 9C). These results demonstrate that not

![Fig. 9. Means ± SE values for mean arterial pressure (MAP) (A), renal vascular resistance (RVR) (B), renal cortical expression of p22\textsuperscript{phox} protein (C), NADPH oxidase activity (D), T\textsubscript{Na}:Q\textsubscript{O2} (E) and renal outer cortical PO\textsubscript{2} (F) in groups of rats infused with vehicle, ANG II (200 ng·kg\textsuperscript{-1}·min\textsuperscript{-1}·sc × 12 days), Tempol (200 nmol·kg\textsuperscript{-1}·min\textsuperscript{-1}·sc × 12 days) or ANG II plus Tempol. Compared with vehicle:*P < 0.05; ***P < 0.005. [From Welch et al. (248).]
only hypertension and renal vasoconstriction but also NADPH oxidase activation and defective oxygenation in the kidney during ANG II can be ascribed to the effects of O$_2$\textsuperscript{−}. There is evidence of bioinactivation of NO by O$_2$\textsuperscript{−} in the kidney of the SHR (253), the postclip kidney of the 2K,1C model (6, 13, 189, 190), and the kidney of the ANG II-infused rat (36). Therefore, enhanced mitochondrial O$_2$ usage during functional NO deficiency as a consequence of oxidative stress could account for the inefficient utilization of O$_2$ by the kidneys of these hypertensive rat models.

Recent studies of isolated mitochondria demonstrate that ROS can interact directly with mitochondrial energy generation (163). ROS, whether produced as a byproduct of mitochondrial respiration or within the cell cytoplasm, can diminish the activity of the mitochondrial uncoupling protein 2 (136). The ensuing proton leak across the mitochondrial membrane reduces the proton motive force that couples O$_2$ usage to ATP synthesis. This provides an additional and more direct mechanism whereby O$_2$\textsuperscript{−} may diminish the efficiency of oxidative metabolism and ATP generation. Indeed, prolonged blockade of ANG II generation or AT$_1$ receptors markedly improves O$_2$ usage and diminishes ROS formation in mitochondria isolated from the kidneys of aged rats (34).

Direct recording of the PO$_2$ in the renal proximal and distal tubules and interstitium suggests that O$_2$ is in diffusional equilibrium within the outer kidney cortex. The highest values of PO$_2$ in the kidney of ~40–45 mmHg are in the outer cortex, yet the PO$_2$ at these sites is below the PO$_2$ of 50–65 mmHg measured in the renal vein (246). This implies a preglomerular diffusional shunt for O$_2$, confirming conclusions from earlier studies (107, 185). This O$_2$ shunt pathway may be between the arcuate arteries and veins, which run a prolonged course in a countercurrent alignment to one another at the junction of the kidney cortex and the outer medulla (95). A shunt that supplies arteriolar O$_2$ to the surrounding tissue, as well as the adjacent arcuate vein, could explain the increase in PO$_2$ at the corticomedullar border, and the relative sparing of this region around the arcuate veins, in a model of ischemic renal injury (171).

The kidneys of rats exposed to hypoxia show a marked accumulation of hypoxia inducible factor-1α and -2α (HIF-1α and -2α). However, these two isoforms are expressed in different cell populations (172). HIF-1α is induced relatively

Fig. 10. Flow diagram of a proposed pathway consequent upon prolonged oxidative stress-induced hypoxia secondary to prolonged ANG II action in the hypertensive kidney.
selectively in tubules, especially the collecting ducts. HIF-2α is not expressed in tubular cells but is detected in some glomerular endothelial cells and more widely in peritubular capillary endothelial cells and fibroblasts (172). The Po2 in the outer cortex is reduced in the kidneys of the SHR (246), the ANG II-infused rat (248), and the postclipped kidneys of the 2K,1C rat (249). The low Po2 and defective TNa:QO2 are normalized by administration of the ARB candesartan in the SHR and by Tempol in the 2K,1C kidney. Thus the efficiency with which the kidney uses O2 for TNa is likely a major determinant of its Po2 and thereby of the expression of HIF-1α and -2α. This efficiency is strongly dependent on ROS.

The effects of ROS to reduce renal parenchymal Po2 could have far-reaching consequences, one of which may be to provide a brake to ongoing oxidative stress (Fig. 10). Thus lipid peroxidation products (132) or O2·− generation from NADPH oxidase (28) increase with O2, yielding an apparent Km for O2 that spans values measured directly in the renal cortex and medulla. Consequently, ROS may inhibit their own production by limiting O2 availability in the kidney, and especially the medulla, of hypertensive animals. However, hypoxia-induced upregulation of HIFs could engage many genes that are implicated in inflammation and fibrosis. This could be a trade-off for the effects of hypoxia to further limit ROS generation (Fig. 10). In this way, hypoxia in the hypertensive kidney could be a switch for a number of potentially adverse mediators. Indeed, hypobaric hypoxia over 24 days to reduce the Po2 by almost 50% not only raises the BP, but leads to renal interstitial inflammation and preglomerular arteriopathy (8).

The expression of HIF-1α in renal medullary interstitial cells in culture is inhibited during coincubation with xanthine and xanthine oxidase to induce O2·−, whereas HIF-1α expression is upregulated by the SOD mimetic Tempol or polyethylene glycol complexed SOD or by blockade of NADPH oxidase with diphenyleneiodonium or apocynin (276). Because scavenging of ·OH with tetramethylthiourea does not change HIF-1α, the authors concluded that O2·−, independent of H2O2 on ·OH−, destabilizes HIF-1α in RMCD cells. Thus there may be two conflicting consequences of an increase in O2·− in the kidney on HIF activity: a direct effect of O2·− to destabilize HIF-1α protein expression and indirect effects mediated by changes in NO activity or mitochondrial function that enhance O2 usage and reduce Po2, thereby increasing HIF-1α generation. Clearly, further studies are required to determine how these discordant
regulatory events play out under conditions of hypertension and renal damage and how important are HIFs for mediating effects of ROS on structural and functional changes in the hypertensive kidney. This may be important because kidneys of rat models of diabetes (94) or chronic renal insufficiency (227) have oxidative stress. Norman and colleagues (45, 140) have proposed that hypoxia in the damaged kidney may activate cytokines that perpetuate renal damage.

CONCLUSION

Growing evidence from animal studies suggests that oxidative stress in the kidney could be a key factor in the development and persistence of hypertension. There is a spectrum of BP responses to antioxidants such as Tempol that range from complete prevention of the development of hypertension in the rat or mouse infused with ANG II at a slow pressor response to partial reversal of established hypertension in the SHR, to more modest antihypertensive effects in stroke-prone SHR. Further work is warranted to translate these findings into clinical investigation and therapeutics. This is of growing importance because all of the established or nontraditional cardiovascular risk factors have been associated in clinical or experimental studies with evidence of increased ROS, which may be linked not only to vasoconstriction, salt retention, and hypertension, but also to many other adverse long-term consequences (Fig. 11; Ref. 19).

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