Renal oxidative stress, oxygenation and hypertension.

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ABSTRACT

Hypertension is closely associated with progressive kidney dysfunction, manifested as glomerulosclerosis, interstitial fibrosis, proteinuria and eventually declining glomerular filtration. The postulated mechanism for development of glomerulosclerosis is barotrauma caused by increased capillary pressure, but the reason for development of interstitial fibrosis and the subsequently reduced kidney function is less clear. However, it has been hypothesized that tissue hypoxia induces fibrogenesis and progressive renal failure. This is very interesting, since recent reports highlight several different mechanisms resulting in altered oxygen handling and availability in the hypertensive kidney. Such mechanisms include decreased renal blood flow due to increased vascular tone induced by angiotensin II that limits oxygen delivery, increased oxidative stress resulting in increased mitochondrial oxygen usage, increased oxygen usage for tubular electrolyte transport and shunting of oxygen from arterial to venous blood in preglomerular vessels. It has been shown in several studies that interventions to prevent oxidative stress and to restore kidney tissue oxygenation prevent progression of kidney dysfunction. Furthermore, inhibition of angiotensin II activity, by either blocking AT₁-receptors or angiotensin converting enzyme, or by preventing oxidative stress by administration of antioxidants also results in improved blood pressure control. It therefore seems likely that tissue hypoxia in the hypertensive kidney contributes to progression of kidney damage and perhaps also maintaining the high blood pressure.

**Key words**: Oxygen consumption, Tubular sodium transport, Blood flow, Oxidative stress, Nitric oxide, Superoxide dismutase.
INTRODUCTION

The kidneys are essential for long-term regulation of arterial blood pressure, as well as for excretion of metabolic waste products and water-soluble toxins. A relatively high renal blood flow (RBF), equal to approximately 25% of the cardiac output at rest, is required for effective clearance of unwanted substances from the blood. Although the kidneys receive a substantial portion of the cardiac output, renal oxygen consumption (QO₂) only equals a few percent of total body QO₂. This results in a surprisingly low oxygen (O₂) extraction and a relatively well oxygenated renal venous blood (22).

Intrarenal blood perfusion is highly heterogeneous, with merely 10% of RBF reaching the deeper situated medullary structures. Interestingly, the heterogeneous RBF distribution and characteristics, in combination with local differences in O₂ demand, result in a distinct intrarenal O₂ gradient (3, 10). The O₂ tension (pO₂) in cortex is about 40-45 mmHg and the lowest renal pO₂ is found in inner medulla (10-15 mmHg) during normal conditions (3). The intrarenal pO₂ values should be compared to that of arterial blood, which is considerably higher (80-100 mmHg). It has therefore been suggested, and to some extent also demonstrated, that O₂ is shunted from arterial to venous blood in preglomerular vessels and vasa recta and as a result never reaches the kidney tissue (3, 19, 63). As a consequence, O₂ delivery to the medulla barely matches demand and relative hypoxia occurs as part of normal kidney function (62). However, the cortico-medullary O₂ gradient is contingent upon prevailing physiological conditions, as evident from the report by Stillman et al. showing that chronic salt depletion for four weeks to rats caused marked cortical hypoxia (112). Furthermore, the medullary oxygenation increased to levels only found in the normally well-oxygenated kidney cortex.

The pO₂ in any tissue is ultimately the relation between O₂ delivery and QO₂. The O₂ delivery to kidney tissue is normally fairly constant, and even if RBF increased dramatically it would likely
also increase QO₂ due to concomitantly increased glomerular filtration rate (GFR), which equals increased tubular sodium (Na⁺) load and therefore also increased QO₂ *per se*. In the normal kidney, approximately 80% of total renal QO₂ is dedicated to electrolyte transport, preferentially consisting of tubular transport of Na⁺ (TNa) (11). Several other O₂-requiring processes also occur in the kidney, e.g. gluconeogenesis, synthesis of glucuronides, serine, acetylated metabolites and hormones. Taken together, available data mainly suggest that QO₂ is the main determinant of intrarenal pO₂ within the normal range of RBF (Fig. 1). Intuitively, one might also consider that increased urinary protein leakage, and thus increased tubular energy demand for protein reabsorption, would contribute to increase kidney QO₂. However, experimental data to support such statements are currently lacking.

Chronic kidney disease (CKD) can be a cause as well as a consequence of hypertension, but blood pressure lowering treatment reduces progression of kidney dysfunction in patients with CKD (35, 72). Hypertension alters intrarenal microcirculation as well as metabolism, and hypertensive kidney damage is a major cause of end-stage renal disease (ESRD). Clinically, hypertensive ESRD is more commonly observed in the presence of atherosclerosis compared to ESRD arising from other etiologies (e.g. polycystic kidney disease, glomerulonephritis, and nephrolithiasis or obstruction) (6). The association of atherosclerosis with hypertensive ESRD presents the possibility that hypertensive ESRD, and possibly even hypertension *per se*, is caused by a primary renal microvascular and/or metabolic disorder (6). Regardless, hypertension constitutes a major risk factor for the progression of renal disease and vascular complications.

This field has been extensively studied to increase the understanding of development and progression of hypertension and its complications. Several animal models are widely used for studying the effects of experimental hypertension, the most common including the spontaneously hypertensive rat (SHR), angiotensin II (Ang II)-induced slow pressor hypertension, 2-kidney, 1-
clip Goldblatt hypertension (2K,1C), Dahl salt-sensitive hypertension, deoxycorticosterone acetate-salt (DOCA-salt) hypertension and the transgenic TG(mREN2)27 rat (Ren2). Hypertensive kidney damage commonly presents as glomerular and tubulointerstitial damage and eventually proteinuria. Lee and co-workers showed that injury or activation of the endothelium by hemodynamic changes resulted in increased local synthesis of angiotensinogen in the remnant rat kidney (60). This initiated a cascade of increased expression of transforming growth factor-β and matrix proteins, which contributed to the development of segmental glomerular lesions. As the development of glomerulosclerosis progresses, endothelial cells decrease due to apoptosis (46), a phenomenon that has been linked to reduced VEGF expression (45). It is now evident that hemodynamic and metabolic factors work together to induce CKD. In addition to glomerular injury, increased vascular tone in renal microvessels limits RBF. Increased Ang II and pressure-induced oxidative stress alter mitochondria and electrolyte transport efficiency, which together reduce kidney pO₂ and can cause tissue hypoxia. Hypoxia is a known stimulus for fibrogenesis, and fibrosis is a common clinical finding in patients with hypertensive kidney damage. Activation of vascular and metabolic pathways that eventually result in hypoxia and interstitial fibrosis are likely to occur long before detectable clinical features of kidney damage are detectable. The role of altered O₂ handling and renal tissue hypoxia for the development of hypertensive kidney damage has recently attracted attention, and the current knowledge is summarized in this review.

**HYPERTENSIVE KIDNEY DAMAGE**

In the USA, hypertension is the second most common cause of ESRD, superseded only by diabetes (28). Johnson et al. suggested division of hypertension into two stages, where the first stage is primarily of non-renal origin but is associated with renal vasoconstriction in the absence
of altered renovascular structure (41). In the second stage, renal vasoconstriction will persist when the external stimuli are removed, likely due to afferent arteriolar damage and interstitial inflammation. As long as glomerular afferent arteriolar structures remain intact, renal autoregulation effectively prevents transmission of increases in systemic blood pressure to renal glomeruli or peritubular capillaries. This is accomplished by two intrarenal mechanisms; the afferent arteriolar myogenic response and tubuloglomerular feedback (TGF). The myogenic response is a reflex causing afferent arterioles to constrict in response to increased arterial pressure. TGF alters afferent tone in response to altered Na$^+$ and Cl$^-$ concentrations in distal tubule as it passes the macula densa. As proposed by Johnson and colleagues, it is in stage two that patients develop salt-sensitivity, renal arteriolar dysfunction and impaired renal autoregulation (41). Renal arteries, including afferent arterioles, undergo pathologic alterations that will compromise autoregulation (48). The endothelium becomes dysfunctional, vasodilatation is gradually impaired and structural changes cause a shift of the autoregulatory curve to a higher set point (40). This stage constitutes a risk for developing microalbuminuria and progressive renal disease that eventually results in ESRD. However, increased oxidative stress induced by Ang II and elevated pressure also induces increased $Q_{O_2}$, resulting in renal tissue hypoxia since increased O$_2$ utilization is not compensated by increased O$_2$ delivery. Sustained hypoxia induces fibrogenesis and tubular atrophy (43), which together with gradual renovascular dysfunction result in progressively diminishing kidney function.

A major part of the hemodynamic, as well as the metabolic alterations in hypertension are connected to reduced kidney tissue pO$_2$. More than twenty years ago, Brazy et al. reported that proximal tubules isolated from early hypertensive SHR display higher $Q_{O_2}$ than tubules from normotensive rats, and tubules from SHR also respond with greater increase in $Q_{O_2}$ when stimulated by norepinephrine (8). Furthermore, tissue pO$_2$ is significantly lower throughout the
kidney in several models of experimental hypertension, including SHR, 2K,1C and Ang II-induced hypertension (82, 83, 123, 125).

When Tigerstedt and Bergman first observed the impressive pressor effects of renal cortical extracts injected intravenously in recipient rabbits, they had just discovered one of the most powerful physiological blood pressure regulating systems; the renin-angiotensin system (RAS) (116). Ang II influences vascular tone via two distinctly different receptors; activation of AT$_1$-receptors causes vasoconstriction, whereas activation of AT$_2$-receptors induces NO release and causes vasodilation. Normally, AT$_1$-receptors are more abundant and constriction therefore dominates the vascular response to exposure to Ang II. Even short term Ang II exposure to both rats and human vascular smooth muscle cells results in contractile dysfunction and induces structural and functional changes in rat kidneys (56, 67). These alterations result in hypertension and vascular and tubulointerstitial damage. Therefore, Ang II is commonly used to induce experimental hypertension, both via exogenous administration, as in Ang II-induced hypertension, and via increased endogenous production, as in 2K,1C. The restriction (clip) placed on the renal artery in the 2K,1C model reduces renal perfusion pressure, which induces renin release (32). This increases Ang II in both kidneys (77) and leads to Ang II-dependent hypertension in rats (98). Furthermore, renal damage and proteinuria are improved by inhibiting Ang II signaling in SHR, but unaffected by similar blood pressure-lowering treatment with the calcium channel blocker amlodipine, (21, 72). Taken together, these reports demonstrate a pivotal involvement of the RAS for development of hypertensive kidney damage.

**DETERMINANTS OF INTRARENAL OXYGEN AVAILABILITY IN HYPERTENSION**

Renal O$_2$ availability is dependent on a balance between delivery and consumption (Fig. 1). Delivery is determined by RBF and O$_2$ extraction, but also by shunting of O$_2$ from arterial to
venous blood in preglomerular vessels. Renal $Q_{O2}$ is affected by mitochondrial function, electrolyte transport and cellular $Q_{O2}$, processes that all can be altered by reactive oxygen species (ROS) in the kidney and renal vasculature. Increased $Q_{O2}$ and oxidative stress are both thought to play important roles in progression of kidney disease, theories supported by the finding that smoking is the strongest independent predictor for decline in renal function in hypertensive patients (90). The rest of this review will mainly focus on these features and on Ang II, nitric oxide (NO), hypoxia-inducible factor (HIF) and other components known to be involved in $O_2$ metabolism and renal function.

**Shunting of oxygen from arterial to venous blood in the renal vasculature**

Despite a high total RBF, the renal cortex displays a relatively low tissue pO$_2$ (about 40-45 mmHg) (3). In two pioneering studies, Levy and colleagues demonstrated the existence of intrarenal shunting of O$_2$ from arterial to venous blood in canine kidneys (63, 64). Labeled erythrocytes and O$_2$ were simultaneously injected into the renal artery whereupon transit time to the renal venous blood was studied. It was shown that O$_2$ could be detected before the labeled erythrocytes appeared. Erythrocytes travel in the intravascular space to end up in venous blood, and since O$_2$ appeared prior to the simultaneously injected erythrocytes (indicating shorter traveled distances), it was concluded that O$_2$ is shunted in the vascular structures (64). Two years later, using a similar experimental approach, Levy and Imperial reported that O$_2$ also is shunted in kidney cortex vasculature (63). In 1990, Schurek *et al.* used micro coaxial needle O$_2$-sensitive electrodes to directly measure glomerular pO$_2$ in 54 anesthetized Munich-Wistar-Fromter rats, and estimated that glomerular pO$_2$ was only half (46±13 mmHg) of that in systemic arterial blood (90±8 mmHg). When rats inhaled 100% O$_2$, arterial pO$_2$ increased more than 6-fold, but glomerular pO$_2$ did not even increase 2-fold. It was suggested that shunting likely takes place
between the countercurrent-arranged interlobular vessels (108). Later, Welch et al. measured local renal pO$_2$ in SHR and Wistar Kyoto rats (WKY) and confirmed shunting in both strains (123). Using Clark-type O$_2$ microelectrodes, they reported consistently lower pO$_2$ in efferent arteriole and cortex compared to that in renal vein, findings that provide further support for a preglomerular O$_2$ shunt.

For years, the significance of intrarenal arterial-venous O$_2$ shunting remained obscure, but recently, Evans and colleagues presented data suggesting that shunting contributes to dynamic regulation of intrarenal pO$_2$ (61). By infusing acetylcholine into rabbit renal arteries the group demonstrated that fractional O$_2$ extraction decreases with increased RBF, but without alterations in renal parenchymal pO$_2$. Hypoxic and hyperoxic ventilation affected renal pO$_2$, but did not change the response to altered RBF. It was concluded that renal tissue pO$_2$ is independent of RBF, a finding that provides further support for that preglomerular shunting regulates renal pO$_2$. However, it should be noted that Welch and colleagues did not report altered shunting of O$_2$ in hypertensive SHR as compared to normotensive WKY (123). It is therefore not likely that differences in O$_2$ shunting contribute to the significantly lower renal tissue pO$_2$ in SHR. However, the role of O$_2$ shunting for regulating intrarenal pO$_2$ in other models of hypertension remains to be determined, especially during situations that could potentially alter the physical driving forces for O$_2$ transport between arterial and venous blood, such as increased diffusion distance due to pronounced fibrosis, or increased diffusion time due to significantly reduced blood perfusion.

**Regulation of basal renal oxygen consumption**

Several factors influence basal renal Q$_{O2}$ (defined as Q$_{O2}$ non-related to tubular electrolyte transport), e.g. NO and Ang II (24, 50, 51). In addition to regulating vascular tone, NO
participates in day-to-day regulation of renal $Q_{O2}$. Acute non-specific inhibition of all nitric oxide synthases (NOS) by L-NAME in dogs resulted in significantly increased renal $Q_{O2}$ although GFR and therefore $T_{Na}$ both decreased (59). Therefore, the effect is likely a direct influence on basal metabolism and mitochondrial function. Similarly, using isolated tubules from rats, Deng and coworkers showed that it is the neuronal NOS isoform that controls proximal tubular $Q_{O2}$ (22). Reduced NO levels increase mitochondrial $Q_{O2}$ and could limit renal tissue $pO_2$. Low NO generation and activity are typical of CKD and kidney injuries are commonly characterized by a NO/$O_2^-$ imbalance (7, 103, 121). By administration of low dose NOS inhibitors (NG-monomethyl-L-arginine and L-NAME) to healthy volunteers and rats, Li et al. recently investigated the effect of NOS inhibition on intrarenal $pO_2$. They measured $pO_2$ and RBF invasively using $O_2$-sensitive optodes and laser Doppler microfibers in anesthetized rats, and $pO_2$ with blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI) in both rats and humans (65). NOS inhibition reduced RBF and caused a dose-dependent decrease in preferentially medullary $pO_2$. Reduced NO bioavailability is suspected in hypertensive kidneys, and a dysfunctional NO balance has been described in SHR, that may contribute to the development of hypertension per se. Although bradykinin, enalaprilat, and amlodipine will decrease kidney $Q_{O2}$ less in SHR compared to kidneys from normotensive rats, SHR kidneys still reduce kidney $Q_{O2}$ in response to NO donors in a similar manner as kidneys from normotensive controls, suggesting the effect is due to decreased NO production rather than to a impaired NO response (1). Ironically, NOS can also contribute to pathophysiological events by magnifying oxidative stress (132). Tetrahydrobiopterin ($BH_4$) is a critical cofactor for NOS, and $BH_4$ oxidation-induced deficiency is manifested as NOS uncoupling. Uncoupled NOS generates $O_2^-$ rather than NO. NOS uncoupling occurs in several disease states characterized by increased oxidative stress, including hypertension. Kidneys of hypertensive Dahl salt-sensitive rats display
NOS uncoupling indicated as L-NAME inhibitable $\text{O}_2^-$ production (114). Similarly, Porkert et al. reported a sustained antihypertensive effect of BH$_4$ administration to patients with poorly controlled hypertension, which was secondary to restored endothelial NO bioavailability (86). Only a minor part of the total kidney $\text{Q}_{\text{O}_2}$ is used for production of $\text{O}_2^-$ and NO in the healthy kidney. It is likely that this contribution would increase during conditions of excessive oxidative stress, such as elevated Ang II levels or inflammation, but this is merely a speculation since there are currently no experimental data available.

Ang II affects renal tissue pO$_2$, partly through effects on NO. Ang II reduced renal pO$_2$ in rats and humans as measured by O$_2$ microelectrodes or BOLD-MRI (83, 102). However, it should be noted that the rapid reduction in pO$_2$ observed in some studies (within 10 seconds of Ang II administration) indicates that reduced RBF rather than increased $\text{Q}_{\text{O}_2}$ is the mechanism altering renal pO$_2$ during acute Ang II administration (102). Ang II-dependent hypertension enhanced $T_{\text{Na}}$-dependent $\text{Q}_{\text{O}_2}$ in thick ascending limb suspensions from rats (111). Recent in vivo data indicated that this effect was NO mediated and independent of renal perfusion pressure (82).

Hypoxia in SHR is tightly linked to Ang II acting on AT$_1$-receptors, since two weeks of treatment with the Ang II AT$_1$-receptor blocker candesartan normalizes renal pO$_2$ (124). The beneficial effect of candesartan is not solely dependent on its blood pressure lowering effects, since pO$_2$ can only be partially restored by lowering the blood pressure to a similar level with combined treatment with the diuretic hydrochlorothiazide, hydralazine and reserpine (124). However, in 2K,1C rats, pO$_2$ in post-clip kidneys is maintained by Ang II acting on Ang II AT$_2$-receptors, emphasizing the importance of intrarenal NO to maintain intrarenal pO$_2$ (82).

Dietary salt intake influences renal pO$_2$. One week of high Na$^+$ intake resulted in decreased medullary pO$_2$, and a week with low dietary Na$^+$ increased pO$_2$ in normotensive men, as well as in untreated hypertensive patients measured by BOLD-MRI (87). In this context, it is worth
noting that Ang II may not only induce hypertension, but also predispose for subsequent salt-sensitive hypertension. After a transient Ang II infusion to rats, Lombardi et al. observed that blood pressure and renal function returned to normal. However, hypertension redeveloped if the previously Ang II-treated rats were again exposed to high Na\(^+\) diet. The authors suggested this may be due to altered renal ability to excrete the elevated salt load, possibly due to peritubular capillary loss coupled with decreased intrarenal NO formation (67). Stillmann et al. reported cortical hypoxia and medullary hyperoxia in chronically salt depleted rats (112). The exact mechanism was not demonstrated, but might include increased Ang II levels to minimize loss of plasma volume and maintain arterial pressure within the normal range.

It has been suggested that hypertension-induced medullary hypoxia may result from hydrogen sulfide (H\(_2\)S) deficiency (4). In mammals, H\(_2\)S is enzymatically generated from L-cysteine or L-homocysteine and acts as a vasodilator via ATP-sensitive potassium channels and therefore regulates vascular tone and blood pressure. In the kidney, H\(_2\)S has been shown to increase GFR and inhibit T\(_{\text{Na}}\). H\(_2\)S is oxidized in mitochondria in a manner dependent on O\(_2\) availability and will therefore accumulate under hypoxia (4). This mechanism may be adaptive as it could potentially increase medullary perfusion and tissue pO\(_2\) in hypoxic states.

**Tubular electrolyte transport efficiency**

Recent studies show that renal hypoxia in hypertension is related to decreased electrolyte transport efficiency, defined as T\(_{\text{Na}}\) achieved with a defined amount of Q\(_{\text{O}_2}\). SHRs are born normotensive, but spontaneously develop hypertension at about five weeks of age. SHR display increased renal vascular resistance resulting in reduced RBF and an enhanced TGF response (123). With O\(_2\) electrodes, Welch et al. demonstrated reduced pO\(_2\) in glomerulus, cortical proximal and distal tubules, as well as in superficial cortical tissue in SHR compared to WKY
rats. It was reported that SHR have reduced RBF, renal O₂ delivery, GFR and T_{Na} compared to WKY. However, total kidney Q_{O₂} was not less in SHR than in WKY rats and the authors therefore concluded that SHR have reduced T_{Na}/Q_{O₂} further limiting renal pO₂ (Fig. 2) (123). Ang II signaling is a likely mechanism for reduced T_{Na}/Q_{O₂} in adult SHR, since treatment with candesartan for two weeks normalized Q_{O₂}/T_{Na} (124). A similar reduction in T_{Na}/Q_{O₂} has also been reported in 2K,1C rats (127). Interestingly, blockade of Ang II AT₁-receptors with candesartan was equally effective as the superoxide dismutase (SOD) mimetic tempol to restore T_{Na}/Q_{O₂} and normalize renal pO₂, highlighting elevated oxidative stress as a common mechanism to induce hypoxia in the hypertensive kidney.

**RENAL PROTECTION AGAINST OXIDATIVE STRESS**

**Superoxide dismutase**

Three different isoforms of SOD catalyze conversion of O₂⁻ to H₂O₂; mitochondrial manganese (Mn)-SOD and two isoforms of copper, zinc-SOD, located either extracellularly (EC-SOD) or intracellularly (IC-SOD) (30, 73). When glutathione is present, catalase or glutathione peroxidase further scavenge H₂O₂ to water. However, in the presence of Fe^{2+} or other trace metals H₂O₂ can decompose to form OH⁻, commonly referred to as the Fenton reaction (26).

Dahl salt-sensitive rats have lower renal SOD and catalase activities, which correspond to elevated oxidative stress (114). The impaired vasodilation in Ang II-induced hypertension is improved by SOD treatment (88). EC-SOD⁻/⁻ mice display increased renal oxidative stress and develop hypertension (126). Interestingly, this is accompanied by reduced pO₂ throughout the kidneys, and both renal hypoxia and hypertension are restored by chronic antioxidant treatment with tempol. Tempol is commonly referred to as a SOD mimetic and reduces any direct effects of O₂⁻, as well as the O₂⁻ driven Fenton reaction (99, 100). In SHR, tempol will normalize blood
pressure, renal vascular resistance, as well as urinary excretion of the marker for elevated oxidative stress 8-Isoprostaglandin F$_{2\alpha}$ (104-106).

In hypertensive rats with chronic renal failure induced by 5/6 nephrectomy, endothelium-dependent relaxation is impaired and O$_2^-$ production increased (133). Chronic treatment with L-arginine, BH$_4$, or SOD all reduced O$_2^-$ production and restored vasorelaxation. The effects were partly additive and it was therefore concluded that both increased NOS uncoupling, resulting in increased radical formation, and decreased NO production, contributed (133).

**Uncoupling proteins.**

Uncoupling proteins (UCP) are proteins expressed in mitochondria and function as proton channels to allow proton leakage back across the inner mitochondrial membrane without creating ATP (36). It has been shown that O$_2^-$ can activate UCP-2 (25, 52), possibly as a protective mechanism against excessive mitochondrial ROS formation in diabetic kidneys (31). However, leakage of protons results in elevated Q$_{O_2}$ to sustain similar ATP production. In 2005, Bernal-Mizrachi et al. demonstrated that mice with inducible UCP-1 expression in aortic smooth muscle cells developed hypertension and arteriosclerosis (5). These findings correlated to increased O$_2^-$ production and decreased NO availability in the vessels.

Recently, Ma and colleagues reported that UCP-2$^{-/-}$ mice developed hypertension when placed on high Na$^+$ diet (8%) for 24 weeks (68). In both wild-type and UCP-2$^{-/-}$ mice the high Na$^+$ diet increased O$_2^-$ production, decreased NO availability, enhanced phenylephrine-induced vasoconstriction and impaired acetylcholine-induced vasodilation. However, these alterations were significantly elevated in UCP-2$^{-/-}$ mice compared to wild-types. The authors concluded that UCP-2 is important for preventing salt-sensitive hypertension, possibly by suppressing O$_2^-$ production and preserving NO bioavailability. Importantly, de Cavanagh et al. showed that
increased H$_2$O$_2$ production, decreased mitochondrial membrane potential and reduced expressions of NOS, Mn-SOD and UCP-2 in SHR mitochondria were due to chronic RAS activation, and not due to the increased blood pressure *per se* (21).

In Dahl salt sensitive rats, where renal SOD is low and superoxide production increased, chronic administration of the antioxidant α-tocopherol (vitamin E) prevented salt-sensitive hypertension and nephropathy (29). In cultured renal tubular epithelial cells, candesartan decreased O$_2^-$ generation and dose-dependently restored redox balance (17). However, similar oxidative stress lowering effects of candesartan were also observed in cultured renal epithelial cells lacking Ang II AT$_1$-receptors. It was concluded that candesartan has a direct antioxidant effect, an effect that was not observed using any of the other highly selective AT$_1$-receptor blockers.

**SOURCES AND TARGETS OF OXYGEN RADICALS IN HYPERTENSION**

ROS are generated by several enzymes as part of normal physiology. Increased production, however, can activate pathological pathways. The most common oxygen radicals are superoxide anions (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radicals (OH$^•$) (49). There are various sources for O$_2$ radical formation. O$_2^-$ is produced by mitochondria as part of normal respiration, as well as by xanthine oxidase, cyclooxygenase, lipoxygenase, uncoupled NOS, cytochrome P450 and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (27). Increased radical formation activates antioxidants to restore redox balance, and it is the balance between ROS production and antioxidant defense mechanisms that determines the oxidative stress status. There are three main targets of O$_2^-$; DNA, protein and lipids. Extensive lipid peroxidation in biological membranes causes alterations in fluidity, permeability and membrane potential, leading to subsequent cell rupture. When proteins are oxidized, their primary structure is altered. Structural alterations include folding as well as charge and hydrophobia and may cause increased
aggregation and degradation. Damage to DNA by ROS includes alterations in DNA structure and chemistry, resulting in strand breakage (20, 39, 70, 94).

Pathological roles of ROS have been established in several diseases, such as ischemia-reperfusion, atherosclerosis, and renal disease (49). Furthermore, excessive oxidative stress is associated with human essential hypertension (55) and documented in several hypertensive animal models, including SHR, 2K,1C, Ang II-induced, Dahl salt-sensitive, and DOCA-salt hypertension (117). Also, there is increasing evidence that oxidative stress not only occurs in parallel with, but also contributes to and accelerates hypertension. It may be that oxidative stress, rather than elevated blood pressure per se is what reduces renal pO₂, and that a defect in renal QO₂ due to oxidative stress may cause exacerbation of hypertension. It should also be noted that elevated perfusion pressure per se can induce vascular ROS production via activation of NADPH oxidase in isolated femoral vessels from rats (120). However, other reports indicate that pressure-induced ROS formation is dependent on Ang II (58). Ang II-induced arterial blood pressure elevation, but not similar increase in blood pressure induced by norepinephrine, resulted in increased vascular ROS formation. Interestingly, hypoxia per se induces increased oxidative stress originating from the mitochondria (12). The mitochondria are able to sustain O₂⁻ production in the nanomolar range also during very low O₂ conditions. However, mitochondrial NO production increases as O₂ is reduced (13), which results in elevated levels of ONOO⁻, the end product when O₂⁻ reacts with NO.

Mitochondria as sources and targets of oxidative stress

Mitochondria are responsible for the majority of cellular energy production, but also modulate apoptosis and H₂O₂ signaling, implying that dysfunctional mitochondria will impair tissue function in several ways. Physiologically, only a small portion of the electrons from the
mitochondrial electron transport chain will reduce O$_2$ to form ROS such as O$_2^-$, a process referred to as leak of electrons. SHR kidneys have increased oxidative stress and concomitant mitochondrial dysfunction manifested as increased H$_2$O$_2$ generation, decreased membrane potential and increased expression of uncoupling protein (UCP)-2 (21). Interestingly, these alterations were prevented by chronic treatment with the Ang II AT$_1$-receptor blocker losartan, which may indicate an additional beneficial mechanism of reducing Ang II signaling in hypertension.

Mn-SOD protects the mitochondria against excessive O$_2^-$, Mn-SOD$^{+/−}$ mice have reduced Mn-SOD levels and develop hypertension with age or when placed on high salt diet (95). Similarly, Ang II-induced hypertension is attenuated in mice over-expressing the human mitochondria-specific antioxidant enzyme thioredoxin 2 (131).

Cardiovascular disease is often accompanied by mitochondrial dysfunction characterized by increased leak of electrons. Ang II is a mediator of renal mitochondrial dysfunction (21), but until recently, the mechanisms remained unknown. In 2008, Dikalov and colleagues investigated the effect of Ang II on mitochondrial respiration, membrane potential, glutathione, mitochondrial ROS and endothelial NO (24). Ang II diminished mitochondrial glutathione, increased state 4 and decreased state 3 respiration and decreased mitochondrial respiratory control ratio by inducing oxidative stress in isolated mitochondria and bovine aortic endothelial cells. These alterations were accompanied by increased mitochondrial H$_2$O$_2$ production, which was inhibited by the NADPH oxidase inhibitor apocynin, a peroxynitrite (ONOO$^-$) scavenger or a protein kinase C inhibitor. These results suggest that Ang II induces mitochondrial dysfunction via a protein kinase C-dependent pathway by activating NADPH oxidase, resulting in increased formation of ONOO$^-$ when the generated O$_2^-$ reacts with NO. Thus, the result would be reduced NO bioavailability in addition to oxidative and nitrosative stress (24). In a more recent study from the
same laboratory (23), it was demonstrated that mitochondria-targeted antioxidants mito-Tempo or mitochondrial Mn-SOD inhibit total cellular $O_2^-$, reduce cellular NADPH oxidase activity and restore NO bioavailability. In the same study it was showed that mito-Tempo prevented hypertension and improved endothelium-dependent vasodilation when given at the onset of Ang II infusion to mice. These results highlight the crucial involvement of mitochondrial $O_2^-$ for the development of hypertension. Notably, in both Ang II-induced and DOCA salt hypertension, blood pressure was lowered by 30 mm Hg by mito-Tempo. Similarly, Mn-SOD over-expressing mice are resistant to Ang II-induced hypertension and vascular oxidative stress (23). If mitochondrial superoxide is involved in hypertension, antioxidants might currently be sub-optimally administered to the sites of superoxide production. These studies suggest that specific targeting of mitochondrial superoxide may be beneficial in treatment of hypertension.

**Renal NADPH oxidase in hypertension**

It is likely that NADPH oxidase and its subunits are pivotal for increasing oxidative stress in the vasculature and kidneys and so make a major contribute to the pathogenesis of hypertension. Ang II acting on AT$_1$-receptors activates NADPH oxidase to generate $O_2^-$, which induces increased expression of the subunits p22$^{\text{phox}}$ and Nox-1 and reduced expression of EC-SOD and Nox-4 (14). However, adverse effects of Ang II via AT$_1$-receptors are to some extent counteracted by AT$_2$-receptor mediated effects, i.e. decreased expression of p22$^{\text{phox}}$, Nox-1, and p67$^{\text{phox}}$ (14). Ang II-induced $O_2^-$ generation by NADPH oxidase results in reduced renal tissue pO$_2$, mainly due to significantly lower $T_{Na}/Q_{O2}$, which is completely prevented by administration of tempol (74, 125). Chabrashvili et al. showed in 2002 that all main components of NADPH oxidase are expressed in SHR kidneys and that there is a prominent increase in p47$^{\text{phox}}$ in vasculature, macula densa, and distal nephron of SHR kidneys compared to WKY controls (15). They also
demonstrated that the increase preceded the development of hypertension. In a subsequent study, the same group reported that high Na\(^+\) intake increased O\(_2\)\(^-\) generation and enhanced renal expression and activity of NADPH oxidase, concomitant with reduced renal expression of intracellular IC-SOD and Mn-SOD (47). Similarly, Adler and colleagues reported Ang II-mediated O\(_2\)\(^-\) production in SHR renal cortex, combined with enhanced expression of NADPH oxidase components and EC-SOD deficiency (1, 2). They suggested that reduced NO bioavailability in the hypertensive SHR kidney results in intrarenal hypoxia, which contributes to renal fibrosis and other injury. Accordingly, increased NO bioavailability, by reducing oxidative stress, would improve renal pO\(_2\). Further support for reduced NO in SHR kidneys is provided by studies reporting that the increased TGF response in SHR is not further elevated by specific neuronal NOS blockade using 7-nitroindazole (128). Antioxidants will reduce blood pressure and renal vascular resistance in SHR. The mechanism likely involves NO since systemic NOS blockade by L-NAME prevents the antihypertensive effect of tempol in this animal model (104). Importantly, tempol as well as the Ang II AT\(_1\)-receptor blocker candesartan restore TGF (128, 129), suggesting that Ang II stimulates ROS generation and reduces NO in SHR. In 2006, Modlinger et al. used sophisticated small interfering RNA *in vivo* against p22\(^{phox}\) in the slow pressor model of Ang II-induced hypertension in rats to markedly reduce blood pressure (74). It is likely that this intervention interfered with normal Ang II signaling to improve renal pO\(_2\). However, a causal link between the improved renal pO\(_2\) and the concomitant reduction in arterial blood pressure is yet to be described, but certainly deserves further attention.

Conscious rats with Ang II-induced hypertension have impaired vasodilation when stimulated by acetylcholine, calcium ionophore A23187, or nitroglycerin (80). Interestingly, Nishiyama and colleagues demonstrated that tempol reduced blood pressure and renal vascular resistance, but only in the presence of NO (80). Furthermore, Welch *et al.* reported that tempol-induced
reduction of oxidative stress in 2K,1C partially corrected renal cortical hypoxia independently of reduction in blood pressure (127). Tempol, but not candesartan, restored pO2 and TNa/QO2 in early 2K,1C. As 2K,1C hypertension progresses, we have reported that the direct Ang II involvement declines and the impact of oxidative stress increases as a result of a self sustained viscous cycle (83). Acute tempol administration still improved blood pressure and RBF more effectively than candesartan in the clipped kidney of twelve month hypertensive 2K,1C rats (83).

Renal O2⁻ is increased in the medulla in Ang II-dependent hypertensive rats (125). Interestingly, Ang II-induced O2⁻ enhanced thick ascending limb TNa related QO2, which was normalized by tempol (111). In mice with Ang II-induced hypertension, NADPH oxidase inhibition blocked O2⁻ generation and lowered blood pressure (92). It has been suggested that part of the Ang II effect on O2⁻ generation is mediated by activation of the immune system. Guzuk et al. reported T-cell activation in Ang II-infused mice, an effect that was abolished in NADPH oxidase-deficient mice (34).

Generation of O2⁻ is also increased in kidneys of hypertensive Dahl salt-sensitive rats on high salt diet. This can be inhibited by L-NAME, suggesting the O2⁻ is due to NOS uncoupling (114). In contrast, L-NAME administered to control rats increased O2⁻ production from the NOS. NOS-related ROS production was increased in aortas of mice with DOCA-salt hypertension (57). Using p47phox⁻/-, nNOS⁻/⁻ and eNOS⁻/⁻ mice, Landmesser et al. demonstrated that DOCA hypertension increased ROS generation from NADPH oxidase, leading to oxidation of BH₄, eNOS uncoupling and further elevated ROS production (57).

A very challenging idea was proposed by Chen et al. in 2005 when they demonstrated that hypoxia per se limits NADPH-dependent O2⁻ production in kidney homogenates from hypertensive SHR as well as normotensive WKY control rats (18). One may speculate that it is
an evolutionary benefit to have a last line of defense against exacerbated ROS production that would otherwise cause acute toxicity.

**CONSEQUENCES OF RENAL HYPOXIA**

Normal kidney function requires an adequate hypoxic gene response to counteract reduced pO₂. Hypoxia-inducible factors (HIF)-1 and -2 are transcription factors that are activated during hypoxia and regulate the hypoxic gene response (109). HIF-1α is degraded by an O₂-dependent mechanism and accumulates during hypoxia to form an active heterodimer with the β-subunit (84). HIF activation has been reported to be beneficial, as well as causative of glomerular injury and renal fibrosis (37, 122). Possibly, HIF activation occurs in parallel with renal injury, but mainly acts to prevent damage. In the kidney, HIF mediates protective pathways, such as erythropoietin, heme-oxygenase (HO)-1 and peroxisome proliferator-activated receptor α-regulated enzyme (16). Activated HIF is also a regulator of several O₂-sensitive genes in the kidney, e.g. NOS and cyclooxygenase-2. However, oxidative stress impairs renal O₂ sensing, as evident from the lack of increased HIF-1α and HO-1 staining in kidneys from streptozotocin-diabetic and Cohen diabetes sensitive rats (96). Furthermore, Katavetin et al. demonstrated that D-glucose, but not L-glucose, significantly blunted hypoxia-induced upregulation of VEGF mRNA in immortalized rat proximal tubular cells (44). Interestingly, they also demonstrated that H₂O₂ blunted this response, whereas α-tocopherol restored the response also during high D-glucose conditions. We recently observed a similar lack of hypoxia-induced increase in VEGF, HO-1 and erythropoietin mRNA levels in kidneys from 4-week streptozotocin-diabetic rats (unpublished results). The lack of activated hypoxic gene response is further supported by the normal or near-normal hematocrits in several hypertensive animal models, including SHR, 2K,1C, Dahl salt-sensitive hypertension and Ang II-induced hypertension, which all have renal
hypoxia. Rosenberger and colleagues showed that antioxidant treatment with tempol to hypoxic kidneys paradoxically increased HIF-1α expression, although tempol reduced tissue hypoxia determined by pimonidazole staining (96). In the absence of an adequate hypoxic gene response to maintain sufficient pO2 in the kidney, the result will be altered salt handling, sustained arterial hypertension, fibrosis and oxidative stress (113). A number of hypoxia-inducing mechanisms have been identified in the tubule, among them increased metabolic demand, insufficient peritubular capillary perfusion due to imbalances in vasoactive substances, and constriction of efferent arterioles due to increased Ang II signaling (9, 107). In addition, Ang II-induced oxidative stress via NADPH oxidase activation will further aggravate hypoxia. Oxidative stress results in inefficient mitochondria respiration, endothelial damage and loss of peritubular capillaries. The result is accelerated hypoxia in the tubulointerstitium (110). Hypoxia will also stimulate regulatory pathways for cellular proliferation and differentiation, and is a powerful stimulus for activation of the immune system to induce differentiation of immature dendritic cells and proliferation of lymphocytes (89). Furthermore, hypoxia-induced HIF activation stimulates target genes, such as vascular endothelial growth factor (VEGF) (89). Interestingly, Rundicki et al. reported that down-regulated VEGF-A predicts progression of proteinuria, renal function, and degree of tubular atrophy and interstitial fibrosis in patients with stable or progressive proteinuric glomerulopathy (97). It should be noted that downregulation of VEGF-A occurred even though the HIF system was activated.

It has been reported that suppression of HIF-1α in the renal medulla of uninephrectomized Sprague-Dawley rats results in salt-sensitive hypertension via dysregulated TNa. Similarly, reduced HIF-1α levels in the renal medulla result in salt-sensitive hypertension also in normal rats (66). Ohtomo et al. reported that induction of HIF-1α with the hypoxia mimetic cobalt
chloride in an obese, hypertensive type 2 diabetic rat model protected kidney function via improvements of proteinuria and histological kidney injury. These improvements were associated with reduced generation of profibrotic factors such as connective tissue growth factor and transforming growth factor-β (53, 54, 81). Uninephrectomized Sprague-Dawley rats transfected with a decoy oligonucleotide inhibiting HIF-1α activity developed hypertension as a consequence of reduced natriuretic response and reduced medullary RBF when challenged with a high Na⁺ diet (66). Taken together, HIF-regulated genes are important determinants of arterial blood pressure in the long-term. By regulating Na⁺ excretion and renomedullary function, HIF-mediated gene activation may act as an antihypertensive pathway. Concomitantly, chronic hypoxia, with or without proper counteracting accumulation of HIF, may induce progressive kidney injury.

**Hemodynamic regulation, kidney function and oxygen**

In rats, neonatal hyperoxic exposure during day 3 to 10 results in potentiated microvascular Ang II-induced contraction and impaired carbachol-induced vasodilation during adulthood (134). These impairments, in conjunction with the reduced nephron number and microvascular rarefaction in the rats exposed to hyperoxia, resulted in significantly elevated arterial blood pressure, implying that hyperoxia interferes with neonatal programming of adult cardiovascular and kidney function.

The majority of data regarding the specific role of O₂ in regulating microvascular tone and kidney function originates from studies using experimental models of chronic hypoxia induced by either hypobaric or normobaric hypoxia. The most commonly used experimental models of hypertension involve administration of exogenous Ang II, or induction of excessive endogenous Ang II production (2K,1C or nephrectomy by ligation of the renal artery), and thus introduce a
major confounding factor since Ang II and the subsequent induction of oxidative stress also impair microvascular and kidney function. However, rats exposed to hypobaric hypoxia for 24 days develop severe arterial hypertension (79). The mechanism responsible for the development of hypertension in their model appears to be reduced NO generation, manifested as reduced urinary excretion of NO metabolites. The hypertension could be prevented by exogenous L-arginine. It is well-known that increased $O_2^-$ scavenges NO, which contributes to the vascular remodeling normally occurring in hypertension (118), but $O_2^-$ will also enhance the myogenic response of the afferent arteriole (91).

The tubular transport of electrolytes requires energy, which is manifested in relatively high kidney $Q_{O_2}$. At the cellular level, reduced $O_2$ availability can reduce ATP production (38) which will affect all energy-requiring processes, including tubular transport. Using mpkCCD-c 14 cells, Husted and co-workers demonstrated a direct regulation of collecting duct epithelial Na$^+$ channels (ENaC) by $O_2$ (38). Reducing $O_2$ levels resulted in reduced ENaC protein levels, as well as reduced ENaC-mediated Na$^+$ transport across the monolayer of the cultured cells. The $O_2$ effects were independent of corticosteroids, and completely opposite to those described in previous reports showing hypoxia-stimulated ENaC expression in rat alveolar epithelial cells (85). Coincidentally, reduced dietary Na$^+$ intake is known to increase medullary pO$_2$ in both rats and humans (87, 112). Thus, the authors speculate that altering the medullary pO$_2$ might be a mechanism for regulating tubular Na$^+$ reabsorption, and therefore be important for maintaining Na$^+$ balance and arterial blood pressure. The reduced renomedullary pO$_2$ in the hypertensive kidney would reduce ENaC-mediated Na$^+$ reabsorption and therefore be a mechanism to counteract the increased blood pressure. This is a speculation that remains to be solidified by experimental data, especially in the view of the very low pO$_2$ required to induce the changes in tubular ENaC levels. However, Gomez et al. (33) showed that increased intrarenal hypoxia and
reduced tubular response to furosemide predicted poor tubular function in poststenotic kidneys. The exact mechanism remains to be determined, but it might involve oxidative stress or hypoxia-induced alterations in the furosemide-sensitive $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ transporter.

**Link between renal hypoxia and fibrosis**

While the body can adapt to short-term hypoxia through induction of HIF-1$\alpha$ expression, chronic hypoxia may induce fibrogenesis. Functional renal impairment correlates with the degree of tubulointerstitial damage and Nangaku et al. proposed that the initial event resulting in kidney failure takes place mainly in the tubulointerstitium (Fig. 3) (75, 76). By exposing renal tubular cells to chronic hypoxia or the hypoxia mimetic cobalt chloride, they showed that HIF activation can induce fibrosis (71). Tubular cells can transform into myofibroblasts, a process commonly referred to as epithelial-mesenchymal transdifferentiation (78). These myofibroblasts act as regulators of renal fibrogenesis, and can induce production of collagen I and $\alpha$-smooth muscle actin expression (71). In addition, transformed cells display increased migratory capacity compared to normal tubular cells. Epithelial-mesenchymal transdifferentiation was confirmed *in vivo* in a rat model of chronic kidney ischemia where the left renal artery was ligated. Thus, Nangaku *et al.* proposed chronic tubulointerstitial hypoxia as a final common pathway to ESRD, suggesting that hypoxia-induced tubulointerstitial damage causes interstitial fibrosis and loss of peritubular capillaries, whereupon fibrosis impairs $\text{O}_2$ supply to tubular and interstitial cells, inducing apoptosis and epithelial-mesenchymal transdifferentiation. It is suggested that this constitutes a vicious cycle that accelerates the development of kidney damage (69, 75, 76, 93). The suggested hypothesis has further support from the study by Higgins *et al.* who reported epithelial-mesenchymal transdifferentiation and epithelial cell migration as a result of HIF activation in primary renal epithelial cells and proximal tubules from mouse kidneys subjected to
unilateral ureteral obstruction (37). Furthermore, inactivation of HIF-1α inhibited tubulointerstitial fibrosis, collagen deposition and decreased inflammatory cell infiltration.

**TREATMENT REGIMENS REDUCING OXIDATIVE STRESS AND HYPOXIA**

Considerable therapeutic effort has been made to prevent or slow progression of hypertension-induced renal disease, and current treatments directed to inhibit Ang II signaling and reduce oxidative stress are indeed renoprotective (42). There is currently no direct evidence for a renoprotective role of the improved renal pO₂ per se when Ang II signaling is inhibited, but this is an area that warrants further attention. Furthermore, treatment with antioxidants has been beneficial for kidney function in a wide range of animal models of experimental hypertension (119). Feeding pregnant SHR and their offspring an antioxidant-enriched diet for 24 weeks, Zhan et al. demonstrated delayed onset and reduced severity of hypertension (135). These reports suggest that not only conventional pharmaceutical interventions, but also modified diet and supplementation with potent antioxidants may attenuate oxidative stress, be renoprotective and reduce the severity of hypertension.

Treatment with 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors, e.g. statins, is common in hypertension. Statins lower serum cholesterol, reduce proteinuria and improve renal function in patients with cardiovascular disease (101). However, in Ren2 rats with an overactivated Ang II system, statin treatment also reduced oxidative stress in kidneys and vasculature with subsequently reduced renal fibrosis (130). Furthermore, statins decreased Ang II-dependent ROS generation, NADPH oxidase activity and expression of several NADPH oxidase subunits in podocytes (130). Since statins normalize endothelium-dependent relaxation even before affecting serum cholesterol levels, it was proposed that beneficial effects of statins are achieved through
improved NO bioavailability and inhibition of $O_2^-$ generation, which perhaps also act to ameliorate renal hypoxia (130).

Finally, directly targeting the chronic renal hypoxia can be a novel and beneficial treatment of hypertensive renal disease. Therefore, non-invasive methods to detect and evaluate the intrarenal $O_2$ availability in affected patients are required. The BOLD-MRI technique measures the level of deoxyhemoglobin as a surrogate for tissue $O_2$ availability. Using BOLD-MRI, Textor et al. demonstrated that interventions to alter intrarenal $Q_{O2}$ can be monitored in patients with renal artery stenosis (115). Similarly, Gomez et al. showed that furosemide-induced reductions in renal $Q_{O2}$ were related to impaired renal function in pigs with renovascular hypertension induced by unilateral renal artery stenosis (33). Animals with intact furosemide-induced suppression of $Q_{O2}$ were normotensive and had GFR comparable to controls. However, animals with reduced furosemide-induced $Q_{O2}$ suppression were hypertensive and had GFR of about 30% of control kidneys. Using computer tomography, the group also reported that pigs with preserved GFR and intact furosemide-induced $Q_{O2}$ suppression displayed a greater increase in tubular fluid concentrating ability compared to those with a suppressed furosemide-induced $Q_{O2}$ response. These studies demonstrate the usefulness of non-invasive imaging techniques to study the link between intrarenal $O_2$ availability and hypertensive kidney damage and how this related to elevated arterial blood pressure.

Renal hypoxia plays a crucial role for development of CKD and ESRD, and potentially also participates in the onset and progression of hypertension, although this has to be further explored. Several pathways can induce excessive $O_2^-$ generation, which is a main mechanism for inducing renal hypoxia. Oxidative stress proceeds hypertension in several animal models of hypertension, and once initiated it can become a self-sustaining ROS generating system (Fig. 4). There is a close relationship between oxidative stress, renal hypoxia and development and progression of
hypertension and hypertensive renal disease, which highlights chronic renal hypoxia as a potential mechanism and a novel therapeutic target to protect the kidney against interstitial fibrosis and perhaps also to counteract hypertension.

PERSPECTIVES AND SIGNIFICANCE

Hypertension is closely associated with increased oxidative stress and reduced pO$_2$ in the kidney, and with increased risk for development of progressive kidney dysfunction. Most clinical and experimental interventions that lower the arterial blood pressure also inhibit oxidative stress and restore kidney pO$_2$, which might contribute to the renoprotection achieved by such interventions. However, there are some questions that still need to be resolved in order to advance the field. 1. Does the reduced kidney pO$_2$ in hypertension contribute to maintaining or accelerating the hypertension? 2. Are kidneys protected from hypertensive damage by selective increases in renal O$_2$ availability? 3. Why does not the reduced pO$_2$ in the hypertensive kidneys result in activation of the HIF system? 4. Is HIF activation protective against hypertensive kidney damage?

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DISCLOSURES

No conflicts of interest are declared by the authors.
REFERENCES


LEGENDS TO FIGURES

**Figure 1.** Factors determining intrarenal oxygen availability.

**Figure 2.** Relationship between tubular sodium transport ($T_{Na}$) and oxygen consumption ($Q_{O2}$) in spontaneously hypertensive rats (SHR) and Wistar Kyoto control rats (WKY). Redrawn from data originally presented by Welch *et al.* (123).

**Figure 3.** Schematic view of the vicious cycle relating tubulointerstitial fibrosis to chronic hypoxia, which creates a self-sustaining mechanism that accelerates the pathological process ending in end-stage renal disease. Redrawn from the hypothesis presented by Nangaku (75, 76).

**Figure 4.** Schematic presentation of the known mechanisms resulting in renal tissue hypoxia and kidney damage in hypertension.
OXYGEN DELIVERY
Determining factors:
Blood flow
Oxygen content of arterial blood
Oxygen extraction
Shunting of oxygen

OXYGEN CONSUMPTION
Determining factors:
Basal metabolism
Electrolyte transport
Efficiency for electrolyte transport
Mitochondrial uncoupling

RENAL TISSUE OXYGEN AVAILABILITY
(pO₂)
Tubulointerstitial injury and fibrosis

- Loss of peritubular capillaries
- Glomerulosclerosis resulting in impaired glomerular capillaries
- Impaired oxygen supply due to increased diffusion distances
- Activation of the renin-angiotensin system

Chronic interstitial hypoxia