Modulation of outer medullary NaCl transport and oxygenation by nitric oxide and superoxide

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Edwards A, Layton AT. Modulation of outer medullary NaCl transport and oxygenation by nitric oxide and superoxide. Am J Physiol Renal Physiol 301: F979–F996, 2011. First published August 17, 2011; doi:10.1152/ajprenal.00096.2011.—We expanded our region-based model of water and solute exchanges in the rat outer medulla to incorporate the transport of nitric oxide (NO) and superoxide (O2) and to examine the impact of NO-O2 interactions on medullary thick ascending limb (mTAL) NaCl reabsorption and oxygen (O2) consumption, under both physiological and pathological conditions. Our results suggest that NaCl transport and the concentrating capacity of the outer medulla are substantially modulated by basal levels of NO and O2. Moreover, the effect of each solute on NaCl reabsorption cannot be considered in isolation, given the feedback loops resulting from three-way interactions between O2, NO, and O2. Notwithstanding vasoactive effects, our model predicts that in the absence of O2-mediated stimulation of NaCl active transport, the outer medullary concentrating capacity (evaluated as the collecting duct fluid osmolality at the outer-inner medullary junction) would be ~40% lower. Conversely, without NO-induced inhibition of NaCl active transport, the outer medullary concentrating capacity would increase by ~70%, but only if that anaerobic metabolism can provide up to half the maximal energy requirements of the outer medulla. The model suggests that in addition to scavenging NO, O2 modulates NO levels indirectly via its stimulation of mTAL metabolism, leading to reduction of O2 as a substrate for NO.

NITRIC OXIDE (NO) and superoxide (O2) exert opposite effects in the renal medulla, and changes in the balance between the two significantly impact renal function. Whereas NO inhibits tubular NaCl reabsorption and enhances medullary blood flow by dilating blood vessels, O2 stimulates NaCl reabsorption across the medullary thick ascending limb (mTAL) and acts to reduce medullary blood flow by mechanisms that remain to be fully elucidated (13).

Under normal conditions, O2 levels in the body are kept low due to O2 scavenging by NO and superoxide dismutase (SOD). As suggested by several studies (reviewed in Ref. 36), an imbalance between NO and O2 in the kidney significantly alters renal hemodynamics and excretory function and may contribute to the development of salt-sensitive hypertension.

An imbalance between NO and O2 may also affect medullary oxygenation. Renal hypoxia is exacerbated during hypertension, and tempol reduces renal tissue hypoxia in spontaneously hypertensive rats (32, 58). Oxidative stress and subsequent reduced NO bioavailability may result in an excessive use of O2 to maintain the sodium balance (i.e., a decrease in TNa/O2, the ratio of transported sodium to oxygen consumption) during hypertension (58).

The objective of the current theoretical study was to investigate how shifts in the balance between NO and O2 affect medullary sodium reabsorption and oxygen availability. We developed a mathematical model of NO and O2 transport in the rat outer medulla to examine the impact of NO-O2 interactions on mTAL sodium transport and O2 consumption, under both physiological and pathological conditions.

MODEL DESCRIPTION

Our representation of the rat outer medulla is that of the region-based approach developed by Layton and Layton (31). The model represents the loops of Henle, the collecting duct (CD) system, the vasa recta, and red blood cells (RBCs). The descending limbs, ascending limbs, and CDs are represented by rigid tubules that are oriented along the corticomedullary axis, which extends from x = 0 at the corticomedullary boundary to x = L at the outer-inner medullary (OM-IM) boundary (Fig. 1A). The model separates blood flow in vasa recta into two compartments, plasma and RBCs, divided by RBC membranes. The vascular plasma and RBC compartments are also represented by rigid tubules along the corticomedullary axis. Besides tubules and vasa recta, the model considers two other sets of compartments: one consists of the RBCs within the capillaries (which we refer to as “capillary RBCs”). Capillary flow is assumed to be perpendicular to the medullary axis; thus the capillary RBC compartment is represented by rigid tubules, extending radially (i.e., perpendicular to the medullary axis) across each medullary level. We assume that the highly fenestrated nature of the capillaries results in rapid equilibration of their plasma content with local interstitium. The other set of compartments represents the combination of interstitial spaces, interstitial cells, and capillary plasma flow, and is simply referred to as the “interstitium.”

The interstitium is divided into four concentric regions, which are used to represent the highly specific structural organization of the rat OM (Fig. 1, B and C): an innermost region containing the central vascular bundle (R1), where all the long descending vasa recta (i.e., DVR that reach into the inner medulla) and a third of the long ascending vasa recta (i.e., AVR that reach into the inner medulla) are sequestered; a peripheral region of the vascular bundle (R2), where the short DVR (i.e., DVR that turn within the OM) and the remaining long AVR reside; a region neighboring the vascular bundle (R3), which contains most medullary thick ascending limbs (mTALs), both long and short, and some short AVR; and

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the region most distant from the vascular bundle (R4), where CDs and the remaining short AVR are located. Descending limbs that reach into the inner medulla are located in R2 and R3 in the outer stripe (OS) and move toward the CDs in the inner stripe (IS). Conversely, the short descending limbs (i.e., those that turn within the OM) straddle R3 and R4 in the OS and move toward the bundle periphery (R2) in the IS.

The model yields tubular and vascular fluid flows as well as the concentration of eight species, i.e., Na\(^+\), urea, O\(_2\), deoxy-hemoglobin (Hb), oxy-hemoglobin (HbO\(_2\)), nitrosyl-heme (HbNO), NO, and O\(_2\), in each type of tubule and vessel, and in the interstitium. These variables are determined based on conservation equations and appropriate boundary conditions. Detailed equations for fluid flows and the concentrations of the first five species (Na\(^+\), urea, O\(_2\), Hb, HbO\(_2\)) can be found in our previous studies (7, 8).

**Conservation Equations**

**Tubules.** Steady-state conservation equations are used to predict the volumetric flow rate \(F_\i\) and the concentration of solute \(k\) \((C_\i\)\) in a tubule of type \(\i\)

\[
\frac{\partial F_\i(x)}{\partial x} = J_{\i,\nu}(x) \tag{1}
\]

\[
\frac{\partial [F_\i(x)C_\i(x)]}{\partial x} = J_{\i,\nu}(x) + \theta A_{\i}\nu(x)[G_{\i}\ep(x) - \Omega_{\i}\nu(x)] - A_{\i}\lum(x)\Omega_{\i}\lum(x) \tag{2}
\]

where \(x\) is the position along the OM, ranging from 0 at the cortico-medullary junction to \(L\) at the OM-IM boundary; \(J_{\i,\nu}\) and \(J_{\i,\k}\) denote the transmural flux of water and solute \(k\) into tubule \(i\); \(A_{\i}\lum\) and \(A_{\i}\ep\nu\) respectively, designate the cross-sectional area of the lumen of \(i\) (i.e., based on its inner diameter) and that of the surrounding epithelium. \(\Omega_{\i}\nu\) and \(\Omega_{\i}\nu\) are the volumetric consumption rate of solute \(k\) in the lumen of tubule \(i\) and that of the surrounding epithelium, and \(G_{\i}\ep(x)\) is the epithelial volumetric generation rate of \(k\). Since the model does not explicitly account for tubular and vascular walls, which are represented instead as single barriers, we assume that a fixed fraction \(\theta\) of the net amount of solute \(k\) that is generated in epithelia or endothelia diffuses toward the lumen, and the remainder \((1 - \theta)\) diffuses toward the interstitium. For NO and O\(_2\), the fraction \(\theta\) is taken as one-half everywhere.

**Vasa recta.** As previously noted, plasma and RBCs are treated as two separate compartments. \(F_{\i}\nu\) and \(F_{\i}\nu\) denote the plasma and RBC water flow rate in vessel \(i\), respectively, so that the total water flow rate in vessel \(i\) is \(F_{\i}\nu\ = F_{\i}\nu\ + F_{\i}\nu\). Similarly, \(C_{\i}\nu\) and \(C_{\i}\nu\) denote the respective plasma and RBC concentration of solute \(k\) in vessel \(i\).

Water conservation in the plasma and RBC compartments of blood vessel \(i\) can be expressed as

\[
\frac{\partial F_{\i}\nu(x)}{\partial x} = J_{\i}\nu(x) \tag{3}
\]

\[
\frac{\partial F_{\i}\nu(x)}{\partial x} = J_{\i}\nu(x) \tag{4}
\]

where \(J_{\i}\nu\) is the net transmural flux of water into plasma (i.e., from the interstitium and RBCs) and \(J_{\i}\nu\) that into RBCs. Note that \(J_{\i}\nu\ = F_{\i}\nu - F_{\i}\nu\), where \(F_{\i}\nu\) designates the transmural flux from interstitium to plasma.

Similarly, solute conservation in the plasma and RBC compartments of blood vessel \(i\) is expressed as

**Fig. 1.** Schematic representation of tubules and vasa recta in the rat outer medulla (OM). Four concentric regions (R1–R4) are distinguished; the innermost (R1) contains the central vascular bundle. Note that R1–R4 have coincident centers; the display here is intended to minimize the figure area. A: tubules and vessels along the corticomedullary axis. B: cross section of the outer stripe. C: cross section of the inner stripe. LDV, long descending vas rectum; SDV, short descending vas rectum; LAVa and LAVb, 2 populations of LDV; SAVa and SAVb, 2 populations of SDV; LDL, long descending limb of Henle’s loop; SDL, short descending limb; LAL, long ascending limb; SAL, short ascending limb; CD: collecting duct. The decimal numbers represent the relative weight of interaction between a type of vessel or tubule and a given region (i.e., the parameter \(\kappa_{i,k}\) in Eqs. 9–10).
\[ \frac{\partial [F^{i}(x)C_{pl}^{k}(x)]}{\partial x} = j^{i}_{l,k}(x) + 0A_{\text{endo}}(x)\left[ G^{\text{endo}}_{i,k}(x) - \Omega^{\text{endo}}_{i,k}(x) \right] \]
\[ - A^{i}_{l,k}(x) \]
\[ \frac{\partial [F^{bc}(x)C_{bc}^{k}(x)]}{\partial x} = j^{bc}_{l,k}(x) - A^{i}_{l,k}(x) \Omega^{bc}_{i,k}(x) \]

where \( j^{i}_{l,k} \) and \( j^{bc}_{l,k} \) are the net transmural flux of solute \( k \) entering plasma and RBCs; \( \Omega^{i}_{l,k}, \Omega^{bc}_{l,k} \), and \( \Omega^{\text{endo}}_{l,k} \), respectively, denote the volumetric consumption rate of solute \( k \) in RBCs, plasma, and surrounding endothelium, and \( G^{\text{endo}}_{i,k} \) is the endothelial volumetric generation rate of \( k \). \( A^{i}_{l,k}, A^{bc}_{l,k} \), and \( A^{\text{endo}}_{l,k} \), respectively, designate the cross-sectional area of the RBC compartment, the plasma compartment, and the surrounding endothelium. The cross-sectional area of the RBC compartment is calculated as:

\[ A^{bc}_{l,k} = A_{i}(F^{bc}_{l,V} + \frac{J^{bc}_{i,k}x}{V^{i}_{bc}}) \]

**Intercellular.** Water conservation equations in the interstitium of each region yield water flows into ascending vasa recta and can be found in our previous study (8). The conservation equation for solute \( k \) in region \( R \) is the interstitial concentration of \( k \), and it is written as:

\[ 2\pi r_{R} \sum_{k \in V_{SA}} P_{R,R_{k}}(C_{R_{k}} - C_{R}) + \sum_{i = \text{SDV,DLV}} n_{i}J_{i,k} + \sum_{i = \text{SDV,DLV}} n_{i}V_{i,k} \]

\[ = C_{AVR, R_{k}} + \sum_{i = \text{SDV,DLV}} n_{i}J_{i,k} + \sum_{i = \text{SDV,DLV}} n_{i}V_{i,k} \]

\[ = C_{AVR, R_{k}} + \sum_{i = \text{SDV,DLV}} n_{i}J_{i,k} + \sum_{i = \text{SDV,DLV}} n_{i}V_{i,k} \]

\[ = C_{AVR, R_{k}} + \sum_{i = \text{SDV,DLV}} n_{i}J_{i,k} + \sum_{i = \text{SDV,DLV}} n_{i}V_{i,k} \]

\[ + \left( 1 - \theta \right) \sum_{i = \text{all tubules}} n_{i}J_{i,k} + \sum_{i = \text{all tubules}} n_{i}V_{i,k} \]

\[ + A_{\text{endo}}^{\text{cap}} C_{cap, R_{k}} - \Omega_{\text{endo}}^{\text{cap}, R_{k}} \]

\[ + A_{\text{endo}}^{\text{cap}} C_{cap, R_{k}} - \Omega_{\text{endo}}^{\text{cap}, R_{k}} \]

\[ + A_{\text{endo}}^{\text{cap}} C_{cap, R_{k}} - \Omega_{\text{endo}}^{\text{cap}, R_{k}} \]

where \( P_{R,R_{k}} \) is the permeability of the boundary between regions \( R \) and \( R_{k} \) to solute \( k \); \( n_{i} \) denotes the number of tubules or vessels of type \( i \); \( \theta \) represents the fraction of short descending vasa recta (SDV) reaching a given medullary region \( R_{k} \); \( Q_{AVR} \) is the capillary flow from region \( R \) to \( R_{k} \), \( Q_{AVR} \) is the total fluid accumulation carried away by AVR, and \( \Omega_{\text{endo}} \) is the area occupied by interstitial cells in region \( R \).

The first term in Eq. 8 represents the diffusion of solute into region \( R \) from adjacent regions \( R_{k} \). The second term is the sum of solute fluxes from tubules and long vasa recta into \( R \). The third and fourth terms denote the composite solute fluxes at level \( x \) from all SDV and short ascending vasa recta (SAV), respectively, that are present in region \( R \) and that reach to medullary level \( y > x \). The first term in the first pair of square brackets represents the solute flux from SDV terminating at level \( y = x \) into region \( R \). The second term in that first pair of square brackets is the solute flux from capillary RBCs into \( R \). The term \( C_{\text{endo}} - \sum_{i = \text{all tubules}} n_{i}J_{i,k} - \sum_{i = \text{all tubules}} n_{i}V_{i,k} \) represents the net amount of solute that is carried by flow at the local concentration into AVR or into an adjoining region \( R_{k} \). The next two terms involving \( 1 - \theta \) represent the fraction of net solute produced by tubular epithelial and vascular endothelial cells that is released into the interstitium (see Eqs. 2 and 5). The next-to-last term denotes the net amount of solute \( k \) produced by capillary endothelial in region \( R \) (see below), and the last term denotes the consumption rate of solute \( k \) by interstitial cells in region \( R \).

**Transmural Fluxes**

**Tubules.** The transmural fluxes of water and solute \( k \) into tubule \( i \) are calculated as:

\[ J_{i,V} = 2\pi r_{i}d_{i} \sum_{k \in \text{solutes}} \kappa_{i,k} \left[ \sum_{k \in \text{solutes}} \sigma_{i,k}(C_{i,k} - C_{R}) \right] \]
Hypoxia-Induced NO Release

As described above, our model does not explicitly represent endothelial and epithelial cell barriers. As shown in Eqs. 2 and 5, we account for NO synthesis (or consumption) in these cellular layers via “source” (or “sink”) fluxes into the vascular or tubular lumen and into the interstitium. Given that solute concentrations in endothelia and epithelia are not explicitly determined, the fraction of NO consumption in these layers that is attributed to plasma or tubular lumen (Eqs. 2 and 5) is calculated based on plasma or luminal concentrations, whereas the fraction that is attributed to the interstitium (Eq. 8) is calculated based on interstitial concentrations.

O2 Generation and Consumption

The rate of epithelial and endothelial O2 synthesis also depends on O2 availability. As we previously described (16), the effects of medullary hypoxia on O2 synthesis remain poorly understood. Given that some studies suggest that low PO2 stimulates O2 synthesis (or consumption) in these cellular layers via the “source” or “sink” reactions with NO and with superoxide dismutase (SOD), we account for this effect in our model of NO transport (16) predicts a 14% increase in medullary NO synthesis in vasa recta is chosen so that predicted values of interstitial O2 are on the order of 1 nM, as discussed below.

The O2 consumption reactions considered here are the scavenging reactions with NO and with superoxide dismutase (SOD). The rate $V_b$ of the latter reaction is calculated as

$$V_{b,5} = k_{SOD}C_{0,2} - C_{SOD}$$

in all compartments (23).

The total volumetric consumption rate of O2 in compartment $i$ is given by

$$\Omega_{i,2-} = V_{i,2} + V_{i,5}$$

As with NO, the fraction of O2 consumed in endothelial or epithelial cells that is attributed to plasma or tubular lumen (Eqs. 2 and 5) is calculated based on plasma or luminal concentrations, whereas the fraction attributed to the interstitium (Eq. 8) is calculated based on interstitial concentrations.

To the best of our knowledge, absolute concentrations of O2 in the medulla have not been reported. We therefore use measurements of its downstream product H2O2, the medullary interstitial concentration of which is ~100 nM (55), to estimate medullary O2 levels. We assume that at steady state, the volumetric generation rate of H2O2 is approximately counterbalanced by its consumption rate by catalase (neglecting diffusion to/from other compartments and other reactions), that is, $0 = \frac{d[H_2O_2]}{dt} = k_{SOD,2-} [SOD] [O_2] - k_{catalase} [CAT] [H_2O_2]$.

The catalase content of the rat liver was estimated as 13 nmol/g wet wt liver (49), that is, ~13 μM. Based upon a kidney-to-liver catalase activity ratio of 0.4 (56), we estimate the renal concentration of catalase to be on the order of 5 μM. Assuming that the intracellular concentration of SOD is 10 μM (18), $k_{catalase} = 3.4 \times 10^7$ M⁻¹·s⁻¹ (42), and $k_{SOD,2-} = 1.6 \times 10^7$ M⁻¹·s⁻¹ (3), Eq. 25 suggests that interstitial O2 concentrations are ~1 nM.

Capillary Endothelial Sources of NO and O2

Recall that the model represents capillaries that traverse radially across the OM cross sections. Since there are very little quantitative data on the medullary capillary network, we assume that capillary plasma is well mixed with the local interstitium. The capillaries thus essentially carry red blood cells. To account for NO and O2 synthesis by the capillary endothelium, we assume that the latter releases NO and O2 directly into the interstitium (which includes capillary plasma). The maximal volumetric generation rate of NO and O2, as well as the endothelial thickness, are taken to be the same in vasa recta and in capillaries. As we also assume that the capillary luminal diameter is 8 μm. Thus the total cross-sectional area of capillary endothelium in a given region R at a given level x along the OM is given by $A_R = n_{cap} \cdot (1 - 0.02SDV/dx) \cdot \pi \cdot (r_{cap} + \delta_{endo})^2 - (r_{cap} - r_{R-1})^2$.

where $n_{cap}$ is the number of capillaries per bundle, $(-0.02SDV/dx)$ is the rate at which SDV break up into capillaries (see Eq. 43 below), $r_{cap}$ is the capillary radius, and $(r_{R-1} - r_{R-1})$ is the distance between the perimeters of regions R and $R-1$.

HbNO Generation and Consumption

HbNO is the product of the reversible reaction between Hb and NO (Eq. 19), and it is sequestered in RBCs. The RBC concentration of HbNO in vessel $i$ is calculated using Eq. 6, with

$$\Omega_{i,HbNO} = -V_{i,4}$$

Active and Basal O2 Consumption

We distinguish between “active” O2 consumption (that is, O2 consumption for active Na⁺ transport), and “basal” O2 consumption (that is, for the basal metabolism of interstitial, endothelial, and epithelial cells). In mTALs, sodium is actively reabsorbed at the
basolateral membrane by Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pumps; given the pump stoichiometry, the number of Na\textsuperscript{+} moles actively reabsorbed per mole of O\textsubscript{2} consumed is taken to be 18 under maximal efficiency. We assume that below a critical P\textsubscript{O\textsubscript{2}} value (denoted P\textsubscript{c}), anaerobic metabolism provides a fraction of the energy needed to actively reabsorb Na\textsuperscript{+}. The volumetric rate of active O\textsubscript{2} consumption in mTAL epithelia (Ω\textsubscript{mTAL,O\textsubscript{2}}) is calculated as

$$\Omega_{\text{mTAL,O}_2}(x) = \frac{2\pi r_{\text{mTAL}}(x) \Psi_{\text{active,mTAL},Na}(x) \Theta\left(P_{\text{mTAL,O}_2}\right)}{18 t_{\text{mTAL}}(x)} \quad (28)$$

where $\Psi_{\text{active,mTAL},Na}$ denotes the mTAL active Na\textsuperscript{+} transport rate, and $\Theta\left(P_{\text{mTAL,O}_2}\right)$ is the fraction of that transport rate that is supported by aerobic respiration, given by

$$\Theta\left(P_{\text{mTAL,O}_2}\right) = \frac{1}{a + (1 - a)\left(P_{\text{mTAL,O}_2}/P_c\right)} \quad (29)$$

The meaning of the parameter $a$ is discussed below.

The volumetric rate of basal O\textsubscript{2} consumption in the epithelium of tubule $i$, or the endothelium of vessel $i$, is calculated as

$$\Omega_{\text{basal,O}_2}(x) = \frac{\Omega_{\text{basal,CNO,Na}}(x)}{C_{\text{O}_2}(x)} \quad (30)$$

The maximal volumetric rate of O\textsubscript{2} consumption (\(\Omega_{\text{basal,O}_2}\)) is assumed to be the same in each compartment and is taken as 10 μM/s (8). To account for the inhibitory effects of NO on mitochondrial utilization, the Michaelis-Menten constant ($K_{\text{M,NO}}$) is taken to vary according to the local NO concentration, i.e.

$$K_{\text{M,NO}}(x) = K_{\text{M,NO}}^0\left[1 + C_{\text{NO}}(x)/C_{\text{inhib}}\right] \quad (31)$$

where $K_{\text{M,NO}}^0$ is the Michaelis-Menten constant in the absence of NO, and $C_{\text{inhib}}$ is the NO concentration that doubles $K_{\text{M,NO}}$; they are respectively taken as 1 mmHg and 27 nM (3).

**Active Na\textsuperscript{+} Reabsorption across mTAL**

The mTAL active Na\textsuperscript{+} transport rate is generally characterized assuming sodium-dependent Michaelis-Menten kinetics

$$\Psi_{\text{active,mTAL},Na}(x) = \frac{V_{\text{max,Na}}(x)C_{\text{mTAL},Na}(x)}{K_{\text{M,Na}} + C_{\text{mTAL},Na}(x)} \quad (32)$$

where $V_{\text{max,Na}}$ (in mol Na\textsuperscript{+}·m\textsuperscript{-2}·s\textsuperscript{-1}) is the maximal rate of Na\textsuperscript{+} transport, and $K_{\text{M,Na}}$ is the Michaelis-Menten constant. The metabolic requirements for this active process are high, and Na\textsuperscript{+} transport may become partly limited by insufficient O\textsubscript{2} availability below the critical P\textsubscript{O\textsubscript{2}} value. In addition, Na\textsuperscript{+} reabsorption across mTALs is inhibited by NO and stimulated by O\textsubscript{2}. As a simplified approach, the effects of oxygen availability, as well as those of NO and O\textsubscript{2}, on $\Psi_{\text{active,mTAL},Na}$ are incorporated separately as follows

$$V_{\text{max,Na}} = V_{\text{max,Na}}^0 \cdot f(C_{\text{mTAL,O}_2}) \cdot g(C_{\text{mTAL,NO}}) \cdot h(C_{\text{mTAL,O}_2}) \quad (33)$$

where $V_{\text{max,Na}}$ is a constant. We assume that below $P_c$, which is taken as 5 mmHg (7), anaerobic metabolism supplies a portion of the energy needed to actively transport Na\textsuperscript{+} across mTALs. More specifically, we assume that in the complete absence of O\textsubscript{2}, anaerobic metabolism produces enough ATP to sustain an active Na\textsuperscript{+} transport rate that is a fraction $a$ (where 0 ≤ $a$ ≤ 1) of the maximum rate when O\textsubscript{2} supply is abundant. With this hypothesis

$$f(C_{\text{mTAL,O}_2}) = \frac{1}{a + (1 - a)C_{\text{mTAL,O}_2}/C_{\text{O}_2}(x)} \quad (34)$$

where $\alpha_{O_2}$ is the O\textsubscript{2} solubility coefficient, taken as 1.34 μM/mmHg. In the base case, $a = 0.5$. A value of 0 means that there is no anaerobic metabolism, and a value of 1 means that anaerobic metabolism can fully sustain the maximal mTAL Na\textsuperscript{+} active transport rate in the absence of O\textsubscript{2}.

Quantitative data regarding the effects of NO and O\textsubscript{2} on NaCl transport across mTALs are very limited. The experiments demonstrating that NO inhibits, and O\textsubscript{2} stimulates, NaCl reabsorption were performed in vitro, where some factors (such as the levels of interacting species) were not controlled. By necessity, the way in which we account for these effects is greatly simplified, and the corresponding parameters are ascribed values that are widely uncertain. To account for the inhibitory effect of NO on $\Psi_{\text{active,mTAL},Na}$ we assume that $V_{\text{max,Na}}$ decreases with increasing NO concentration according to

$$g(C_{\text{mTAL,NO}}) = 1 - \frac{C_{\text{mTAL,NO}}}{\beta + C_{\text{mTAL,NO}}} \quad (35)$$

Ortiz et al. (48) reported that 10 μM spermine NONOate (or SPM, an NO donor) inhibits mTAL Cl\textsuperscript{-} reabsorption by 46%. At a concentration of 10 μM, SPM is expected to result in a bath concentration of 50–60 nM NO (51). Using these data, the constant $\beta$ is estimated as 46.9 nM.

It has been shown that endogenously produced O\textsubscript{2} stimulates mTAL NaCl transport independently of NO. In the absence of l-arginine, the O\textsubscript{2} scavenger tempol (50 μM) was found to decrease mTAL Cl\textsuperscript{-} reabsorption by ~30% after 20-min incubation (46). Since the first-order rate constant for the dismutation of O\textsubscript{2} by tempol is 6.5 × 10\textsuperscript{4} M\textsuperscript{-1}·s\textsuperscript{-1} (29), the concentration of O\textsubscript{2} after a 20-min equilibration with tempol should be vanishingly small, and we assume that reducing the mTAL concentration of O\textsubscript{2} from a reference value (C\textsubscript{mTAL,O\textsubscript{2}}\textsuperscript{0}) to zero decreases NaCl active transport by 30%. Specifically, we assume that $V_{\text{max,Na}}$ increases with increasing O\textsubscript{2} concentration according to

$$h(C_{\text{mTAL,O}_2}) = 0.7 + 0.6\left(\frac{C_{\text{mTAL,O}_2}}{C_{\text{mTAL,O}_2}^0 + C_{\text{mTAL,O}_2}^0}\right) \quad (36)$$

The reference values (C\textsubscript{mTAL,O\textsubscript{2}}\textsuperscript{0}) are chosen so that $h \sim 1$ in the basal configuration. Based upon preliminary simulations, C\textsubscript{mTAL,O\textsubscript{2}}\textsuperscript{0} is taken as 20 pM in case A and 350 pM in case B. The constant in the vessel of NO and O\textsubscript{2} effects (31). To obtain a two- to threefold increase in the osmolality of the CD fluid between the corticomedullary junction and the boundary between the outer and inner medulla, those V\textsubscript{mTAL} values are multiplied by 2.2.

**Permeability to NO and O\textsubscript{2}**

Aquaporin-1 (AQP1) water channels have been shown to transport NO and O\textsubscript{2} (21). As previously described (16), we use the empirical correlation obtained by Herrera et al. (21) to estimate the permeability of tubule or vessel $i$ (P\textsubscript{NO}, P\textsubscript{O\textsubscript{2}}) to NO, given the basal RBC permeability to NO (P\textsubscript{NO,RBC,basal})

$$P_{i,NO} = P_{b,RBC,basal} \left(\frac{0.64P_i + 20.23}{0.64P_i + 20.23}\right) \quad (37)$$

where $P_i$ is the water permeability of tubule or vessel $i$. In the vessels and tubules that do not express AQP1 (i.e., AVR, ascending limbs, and CDs) $P_{i,NO}$ is taken as zero in Eq. 37.

Even though the lipid bilayers are almost impermeable to O\textsubscript{2} (19), chloride channels have been shown to mediate O\textsubscript{2} transport in endothelial cell plasma membranes (43). In the absence of more specific data, we assumed that the permeability of OM tubules and vessels to O\textsubscript{2} is 5 × 10\textsuperscript{-4} cm/s.

The effective permeability to solute $k$ (k = NO, O\textsubscript{2}) of the boundary separating regions R and R' is estimated as
The tortuosity of the proximal straight tubules. *Ascending vasa recta (AVR) water permeability is not given because AVR water fluxes are computed based on fluid accumulation into a region.

\[
p_{k, R}^{R} = \frac{A_{F, R}^{R}}{\gamma d_{R, R}^{R}} \quad k = \text{NO}, \text{O}_2^- \quad (38)
\]

where \(A_{F, R}^{R} \) is the fraction of the \( R-R' \) interface available for interstitial diffusion, \( D_k \) is the diffusivity of solute \( k \) in dilute solution, and \( d_{R, R}^{R} \) is the distance between the midpoints of regions \( R \) and \( R' \). The parameter \( \gamma \) is a diffusion resistance which accounts for the hindering effects of macromolecules and cells in the interstitium. We assume that \( \gamma \) equals 5 for both NO and \( \text{O}_2^- \), based upon measured membrane-to-dilute solution NO diffusivity ratios (15). The factor \( \tau \) represents the effect of tortuosity on the diffusion path length around tubules and vessels and is taken as \( \pi/2 \) (31). The diffusivity of NO and \( \text{O}_2^- \) in dilute solution is taken as 3,300 and 2,800 \( \text{m}^2/\text{s} \), respectively (6, 38).

Boundary Conditions

The boundary conditions for the flows of water, \( \text{Na}^+ \), urea, \( \text{O}_2 \), \( \text{Hb} \), and \( \text{HbO}_2 \) were described previously (8). The concentrations of NO and \( \text{O}_2^- \) are specified at the corticomedullary junction in descending vessels and tubules (Table 1). They must also be prescribed in long ascending and descending limbs, and in long ascending and descending vasa recta. Thus the concentration of solute \( k (k = \text{NO} \) and \( \text{O}_2^- \) \) in LAL fluid and LAV plasma at \( x = L \) is obtained by solving the following equations.

**Long ascending limbs.**

\[
\sum_{j=a, b} n_{LAL, j} F_{LAL, j}^{pl}(L) C_{LAL,j}^{pl}(L) = n_{LDL, j} F_{LDL, j}^{pl}(L) C_{LDL,j}^{pl}(L) \quad (39)
\]

**Long vasa recta.**

\[
\sum_{j=a, b} n_{LAV, j} F_{LAV, j}^{pl}(L) C_{LAV,j}^{pl}(L) = n_{LDF, j} F_{LDF, j}^{pl}(L) C_{LDF,j}^{pl}(L) \quad (40a)
\]

\[
C_{LAV,j}^{pl}(L) = C_{LAV,j}^{pl}(L) \quad (40b)
\]

We also assume that the RBC-to-plasma concentration ratio for solute \( k (k = \text{NO} \) and \( \text{O}_2^- \) \) is the same in long ascending and descending vasa recta at \( x = L \).

Table 1. Vessel and tubule dimensions

<table>
<thead>
<tr>
<th>Vessel or Tubule</th>
<th>Inner Diameter, ( \mu )m</th>
<th>Endothelial/Epithelial Layer Thickness, ( \mu )m</th>
<th>Permeability to Water, ( \mu )m/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long and short descending vasa recta</td>
<td>11</td>
<td>1.0</td>
<td>1,257</td>
</tr>
<tr>
<td>Long and short ascending vasa recta</td>
<td>28 → 10</td>
<td>1.0</td>
<td>*</td>
</tr>
<tr>
<td>Long descending limbs</td>
<td>80 in OS</td>
<td>1.1</td>
<td>3,570 in OS</td>
</tr>
<tr>
<td></td>
<td>21 → 16 in IS</td>
<td>1.1</td>
<td>2,295 in IS</td>
</tr>
<tr>
<td>Short descending limbs</td>
<td>21 → 16</td>
<td>1.1</td>
<td>3,257 in IS</td>
</tr>
<tr>
<td>Long ascending limbs</td>
<td>20</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>Short ascending limbs</td>
<td>21 → 10</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>Collecting ducts</td>
<td>31 → 22</td>
<td>9.0</td>
<td>463</td>
</tr>
</tbody>
</table>

Diameters and water permeabilities are taken from Ref. 31, thicknesses from Ref. 8. IS, inner stripe. The arrow indicates that the parameter value decreases linearly along the \( x \)-axis (from \( x = 0 \) to \( x = L \), unless indicated otherwise). The large diameter of the long descending limbs in the outer stripe (OS) represents the tortuosity of the proximal straight tubules. *Ascending vasa recta (AVR) water permeability is not given because AVR water fluxes are computed based on fluid accumulation into a region.

\[
C_{LAV,j}^{pl}(L) = C_{LAV,j}^{pl}(L) \quad (41)
\]

The RBC concentration of \( \text{HbNO} \) is specified as 1 \( \mu \)M in descending vasa recta at the corticomedullary junction. This estimate is in the midrange of reported values (24, 30). At the boundary between the outer and inner medulla, the molar flow rate of \( \text{HbNO} \) leaving LDV is taken to be equal to that entering LAV

\[
\sum_{j=a, b} n_{LAV, j} F_{LAV, j}^{pl}(L) C_{LAV,j}^{pl}(L) = n_{LDF, j} F_{LDF, j}^{pl}(L) C_{LDF,j}^{pl}(L) \quad (42a)
\]

\[
C_{LAV,j}^{pl}(L) = C_{LAV,j}^{pl}(L) \quad (42b)
\]

**Parameter Values**

The dimensions of tubules and vessels are given in Table 1, those of interstitial regions in Table 2. Shown in Table 3 are NO and \( \text{O}_2^- \) generation rates, permeabilities, and inlet boundary conditions. Kinetic parameters are listed in Table 4. The fraction of short vasa recta present at level \( x \) is given by

\[
\omega_{SDV}(x) = \begin{cases} 1 - 0.1x/L & 0 \leq x \leq L_{OS} \\ (1 - 0.1L_{OS}/L)(1 - x/L) & L_{OS} \leq x \leq L \end{cases} \quad (43)
\]

where \( L_{OS} \) is the outer-stripe length (0.6 mm), and \( L \) is the total OM length (2.0 mm).

**Numerical Methods**

The steady-state differential equations are discretized to form a system of nonlinear algebraic equations. A spatial discretization of 200 grid points along the medullary axis and 50 grid points in the radial direction is used. The system of coupled, nonlinear conservation equations is solved using MINPACK, a numerical package implemented in FORTRAN for solving nonlinear equations with a modification of Powell’s hybrid algorithm, in an iterative approach described in a previous study (8).

Table 2. Region parameters

<table>
<thead>
<tr>
<th>Region radii, ( \mu )m</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial areas, ( \times 10^{-6} ) cm(^2)</td>
<td>2.41–2.26</td>
<td>87.8–16.2</td>
<td>126–137</td>
<td>53.2–91.6</td>
</tr>
<tr>
<td>Fractional area available for diffusion, ( A_F, R, R+1 )</td>
<td>0.0560–0.0398</td>
<td>0.0560–0.120</td>
<td>0.0560–0.199</td>
<td></td>
</tr>
<tr>
<td>Area of capillary endothelial compartment, ( \times 10^{-6} ) cm(^2)</td>
<td>0.0424–0.666</td>
<td>0.435–2.47</td>
<td>0.284–2.20</td>
<td>0.0924–1.10</td>
</tr>
</tbody>
</table>

The first value corresponds to the corticomedullary junction, the second to the mid-IS. Except for capillary endothelial areas, which are given by Eq. 26, the data are taken from Ref. 31.
Table 3. NO and O2 transport parameters

<table>
<thead>
<tr>
<th>Parameter Definition</th>
<th>Descending Vasa Recta</th>
<th>Ascending Vasa Recta</th>
<th>Descending Limbs</th>
<th>Ascending Limbs</th>
<th>Collecting Ducts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal epithelial/endothelial NO generation rate, $G_{NO}^{max}$, $\mu M \cdot s^{-1}$</td>
<td>76.6</td>
<td>72.2*</td>
<td>12.3</td>
<td>0.985</td>
<td>0.415</td>
</tr>
<tr>
<td>Epithelial/endothelial O2 generation rate, $G_{O2}^{gen}$, $\mu M \cdot s^{-1}$</td>
<td>21.0</td>
<td>19.8*</td>
<td>15.2</td>
<td>3.2</td>
<td>1.8</td>
</tr>
<tr>
<td>NO permeability†, cm/s</td>
<td>0.496</td>
<td>0.0122</td>
<td>0.896 $\rightarrow$ 1.387‡</td>
<td>0.0122</td>
<td>0.0122</td>
</tr>
<tr>
<td>O2 permeability‡, cm/s</td>
<td>0.0005</td>
<td>0.0005</td>
<td>0.0005</td>
<td>0.0005</td>
<td>0.0005</td>
</tr>
<tr>
<td>NO concentration at inlet, nM</td>
<td>RBC: 0.1</td>
<td>N.A.</td>
<td>100</td>
<td>NA</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Plasma: 100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O2 concentration at inlet, nM</td>
<td>RBC: 0.1</td>
<td>0.02</td>
<td>NA</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma: 0.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NO, nitric oxide; O2, superoxide; NA, not applicable. *The AVR rate is obtained by multiplying the DVR rate by $(1 - f_{fen})$, where $f_{fen}$ is the fraction of the AVR surface that is fenestrated (taken as 0.057, based on Ref. 35). †Permeability values correspond to the vascular or tubular wall. The red blood cell (RBC) permeability to NO is taken as 0.1 cm/s in the base case. ‡Depending on the descending limb portion (note that the NO permeability scales with water permeability).

RESULTS

Base-Case Description

In baseline simulations, the RBC permeability to NO is kept constant, and the O2 generation rate is taken to vary in parallel with PO2 (case A). In other words, the base case does not incorporate hypoxia-induced increases in NO release and in O2 generation. These effects are examined further below.

PO2 profiles are similar to those described in our recent studies (7). Briefly, our model predicts that the OM architecture results in significant PO2 gradients both in the axial and the radial directions (Fig. 2A). The segregation of long DVR at the center of the vascular bundles (in region R1) preserves enough O2 for delivery to the inner medulla (IM), while the high metabolic requirements of mTALs in the peripheral regions (R2–R4) significantly deplete O2 therein.

NO concentration (CNO) profiles are shown in Fig. 3. As described in our previous model of transport in cross sections of the rat OM (16), the large radial PO2 gradients in turn generate substantial radial NO and O2 concentration gradients, since the synthesis of both solutes is O2 dependent. Vasa recta endothelial cells are far by far the largest source of NO, but RBCs constitute a sink for NO because hemoglobin scavenges NO at a very rapid rate ($\sim$ 100 times faster than O2 does), and the RBC permeability to NO is relatively high. Thus, without the rate-limiting effects of O2 on NO generation, CNO would be lowest in the vascular bundle core, that is, the region with the highest density of RBCs. When those rate-limiting effects are taken into account, CNO is predicted to be significantly higher in R1 (the region with the highest PO2) than in R2 and R3 (the regions with the poorest O2 availability) in the inner stripe (Fig. 3E).

In the outer stripe (OS) of the OM, several classes of tubules straddle two peripheral regions (see Fig. 1B), leading to significant mixing between R2, R3, and R4. Thus interstitial CNO is very similar in those three regions. It is slightly higher in R4, a region with relatively high PO2 levels and few vessels (i.e., few NO sinks). In the inner stripe (IS), the external regions are more segregated, leading to sharper CNO differences between regions.

The sharp rise in interstitial CNO in R4 at the OS-IS junction is caused by tubule migration. As illustrated in Fig. 1, the short descending limbs are near CDs in the OS (i.e., they straddle R3 and R4), but move to the immediate periphery of the vascular bundle (i.e., to R2) in the IS. These short (and thin) descending limbs generate less NO than the other tubular and vascular structures in R4. Thus, in the OS, NO diffuses from the R4 interstitium into their lumen, and when this flux abruptly ceases at the OS-IS junction, interstitial CNO increases sharply in R4. If the position of these short descending limbs were to be maintained constant in the OM, there would be no such sharp increase (results not shown). In the deep IS, R4 PO2 drops significantly, and so does the interstitial CNO in R4.

In R2 and R3, by contrast, interstitial CNO drops suddenly at the OS-IS junction. Indeed, due to an increase in the maximal rate of Na+ active transport across mTALs at the junction, PO2 levels fall significantly in R2 and R3 (see Fig. 2A), thereby greatly reducing NO generation in those regions. In addition, long descending limbs are taken to be very large in the OS (to account for the tortuosity of proximal tubules), and thus constitute a significant source of NO therein; their diameter decreases sharply at the OS-IS junction, which further reduces NO generation in R2–R3. Even though PO2 is higher in R2 (where there are fewer mTALs), interstitial CNO is higher in R3 than in R2 mainly because of the short ascending limbs: the latter tubules straddle R3 and R4 and receive a substantial amount of NO from R4, which then diffuses from the short ascending limb lumen in the R3 interstitium. Note that along the corticomedullary axis, and within each stripe, CNO generally rises and falls with PO2.

Superoxide concentration (CO2−) profiles are illustrated in Fig. 4. There is as yet no evidence that AQP1 is permeable
to $\mathrm{O}_2^-$, and vascular and tubular permeabilities to $\mathrm{O}_2^-$ are taken to be significantly lower than those to NO. Thus transmembrane $\mathrm{C}_{\mathrm{O}_2^-}$ gradients are predicted to be significant, and in a given region, $\mathrm{C}_{\mathrm{O}_2^-}$ is significantly higher in the interstitium than in tubular lumen or plasma. In the base case, the $\mathrm{O}_2^-$ generation rate is assumed to decrease with decreasing $P_{\mathrm{O}_2}$, and close examination of the curves reveals that interstitial $\mathrm{C}_{\mathrm{O}_2^-}$ profiles closely track $P_{\mathrm{O}_2}$ profiles. Note that $\mathrm{C}_{\mathrm{O}_2^-}$ is more elevated in R1 than in the peripheral regions not only because $P_{\mathrm{O}_2}$ is higher in the vascular bundle core but also because the model postulates that there is much less water accumulation, and therefore less dilution, therein. The sharp decrease in interstitial $\mathrm{C}_{\mathrm{O}_2^-}$ at the OS-IS junction in the interbundle region stems from the sudden diminution in the long descending limb diameter in R2 and R3, as well as the migration of short descending limbs out of R4 (Fig. 4).

One measure of the concentrating capacity of the OM is the osmolality of the tubular fluid in the CD at the OM-IM junction (denoted $\mathrm{osm}_{\mathrm{CD}}$ hereafter). Under basal conditions, the latter equals 787 mosmol/kgH$_2$O (Table 5).

**Effects of Hypoxia**

As described above, the mechanisms by which hypoxia modulates medullary NO and $\mathrm{O}_2^-$ levels remain uncertain. To incorporate the effects of hypoxia on $\mathrm{C}_{\mathrm{NO}}$ in a simple manner, we assumed that the RBC permeability to NO ($P_{\mathrm{NO}}^\text{rbc}$), and thus the strength of the RBC sink, decrease with decreasing $P_{\mathrm{O}_2}$ (Eq. 21). With this hypothesis, interstitial $\mathrm{C}_{\mathrm{NO}}$ is predicted to rise significantly relative to the base case in the peripheral, $\mathrm{O}_2$-starved regions (R2–R4), and to decrease in the central, $\mathrm{O}_2$-rich region (R1), as displayed in Fig. 5. Higher NO levels in mTALs result in greater inhibition of NaCl reabsorption ($G_{\mathrm{mTAL}, \mathrm{NO}}$) (Eq. 33). In the absence of NO scavenger, hemoglobin, is the least predominant. In that case, $\mathrm{osm}_{\mathrm{CD}}$ is predicted to decrease by 20%, from 787 to 714 mosmol/kgH$_2$O (Table 5).

**NO-Mediated Inhibition of mTAL Sodium Reabsorption**

To determine the extent to which NO-induced inhibition of mTAL NaCl reabsorption affects the concentrating capacity of the OM and its oxygenation, we performed simulations in which NO effects on NaCl transport were abolished. That is, we set $g(C_{\text{mTAL}, \text{NO}}) = 1$ in Eq. 33. In the absence of NO-mediated inhibition, the rates of NaCl reabsorption and $\mathrm{O}_2$ consumption are predicted to both rise markedly relative to the

---

**Fig. 2. Oxygen tension ($P_{\mathrm{O}_2}$) profiles in the interstitium of the 4 regions (R1–R4) in the base case (A), assuming that nitric oxide (NO) does not inhibit medullary thick ascending limb (mTAL) NaCl reabsorption (B), and assuming that $\mathrm{O}_2$ does not stimulate mTAL NaCl reabsorption (C). x/L denotes the ratio of the axial coordinate to total length of outer medulla. In all cases, the red blood cell (RBC) permeability to NO ($P_{\mathrm{NO}}^\text{rbc}$) is kept constant, and the $\mathrm{O}_2$ generation rate ($G_{\mathrm{O}_2^-}$) is taken to vary in parallel with $P_{\mathrm{O}_2}$.**
base case. In R2 and R3, PO2 drops below the critical pressure throughout most of the IS (Fig. 2B). The rate of NaCl active transport can nevertheless increase substantially because of anaerobic metabolism. The model predicts that the concentrating capacity of the OM rises by 70%, that is, osmCDL increases from 787 to 1,333 mosmol/kgH2O (Table 5). In the vascular bundle core (R1), PO2 remains relatively unchanged and oxygen delivery to the IM is preserved.

Since NO generation decreases with decreasing PO2, CNO is then significantly lower in R2–R4, relative to the base case (Fig. 8). Under these conditions, CNO remains relatively unchanged and oxygen delivery to the IM is preserved.

Similar trends are obtained if we assume that the RBC permeability to NO decreases with decreasing PO2, as described by Eq. 21. Eliminating NO-mediated inhibition of mTAL transport raises osmCD by 80% in that case (Table 5). Even though the PO2 drop in R2–R4 reduces RBC removal of NO therein, the concomitant decrease in NO generation predominates and CNO is also predicted to decrease in the peripheral regions under this assumption (results not shown).

Given that NO scavenges O2-, an isolated reduction in CNO should raise CO2-. However, assuming that O2- generation decreases with decreasing PO2, the reduction in O2- consumption is accompanied by a greater reduction in O2- production, and CO2- is predicted to decrease in R2–R4 when NO-mediated inhibition of mTAL transport is eliminated (Fig. 9). The decrease is more pronounced in the OS (20–50%) than in the IS (10–20%) because PO2 drops more sharply in the upper OM. Conversely, if we were to assume fixed O2- generation rates, CO2- would increase by ~5% in the peripheral regions in the absence of NO effects on NaCl reabsorption, given the concomitant reduction in the O2 consumption rate (results not shown).

O2- Mediated Activation of mTAL Sodium Reabsorption

As opposed to NO, O2- stimulates NaCl reabsorption across the mTAL. In the next set of simulations, we removed these O2- -induced effects to assess their importance. That is, we set h(CmTAL, NO) = 0.70 in Eq. 33.

In the absence of O2- -mediated stimulation of active transport, the rate of O2 consumption diminishes, interbundle PO2 levels increase, and so does NO production in R2–R4. Thus NO-induced inhibition of mTAL active transport rises in turn, thereby increasing PO2 levels further and exerting a positive feedback loop. Nevertheless, the limited O2 supply to the interbundle region halts the PO2 and NO increase therein.
Given this positive feedback loop, the concentrating capacity of the OM is predicted to be significantly lower relative to the base case: osmCD is then equal to 493 (vs. 787) mosmol/kgH2O (Table 5).

Table 5. Effects of NO and O2 on the concentrating capacity of the OM

<table>
<thead>
<tr>
<th></th>
<th>Case A</th>
<th>Case B</th>
<th>Case C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td>787</td>
<td>878</td>
<td>729</td>
</tr>
<tr>
<td>Without the NO/O2 reaction</td>
<td>770</td>
<td>714</td>
<td>708</td>
</tr>
<tr>
<td>Without NO-mediated inhibition of mTAL active transport</td>
<td>1,333</td>
<td>1,398</td>
<td>1,328</td>
</tr>
<tr>
<td>Without O2-mediated stimulation of mTAL active transport</td>
<td>493</td>
<td>584</td>
<td>453</td>
</tr>
</tbody>
</table>

osmCD, osmolality of the collecting duct (CD) tubular fluid at the boundary between the outer (OM) and inner medulla; case A, O2 synthesis is taken to decrease with decreasing PO2 (Eq. 22a), and the RBC permeability to NO (PNO) is taken to remain constant; case B, O2 synthesis is taken to increase with decreasing PO2 (Eq. 22b), and PNO is taken to remain constant; case C, O2 synthesis is taken to decrease with decreasing PO2 (Eq. 22a), and PNO is taken to vary with PO2 (Eq. 21); mTAL, medullary thick ascending limb.

Without O2-mediated stimulation of NaCl reabsorption, PO2 is predicted to hover above 20 mmHg in all regions all the way down to the mid-IS (Fig. 2C). With or without hypoxia-mediated effects on NO release, interstitial CNO is then predicted to remain higher in R3–R4 than in R1 throughout most of the medulla (Fig. 8). Indeed, as noted above, without the rate-limiting effects of O2 on NO generation rates, CNO is predicted to be the lowest in the vascular bundle core, where the relative density of Hb-carrying blood vessels is the highest.

When O2 effects on NaCl transport are eliminated, the PO2 elevation translates into an increase in O2 generation, assuming that O2 generation increases in parallel with PO2, so that interstitial CNO is then predicted to remain higher in R3–R4 than in R1 throughout most of the medulla (Fig. 8). Indeed, as noted above, without the rate-limiting effects of O2 on NO generation rates, CNO is predicted to be the lowest in the vascular bundle core, where the relative density of Hb-carrying blood vessels is the highest.

Contribution of Anaerobic Metabolism

In all the preceding simulations, we assumed that glycolysis provides a substantial fraction of the energy needed to
actively reabsorb NaCl across mTALs when P<sub>O2</sub> drops below the critical pressure. How would our predictions differ in the absence of anaerobic metabolism (i.e., if the parameter <i>a</i> in Eqs. 28 and 29 were equal to 0 instead of 0.5)? The active transport rate would then be considerably limited by the hypoxic conditions that prevail in the renal medulla, and all else being equal, the concentrating capacity of the OM would greatly diminish.

Fig. 5. C<sub>NO</sub> profiles in the interstitium of the 4 concentric regions (R1–R4). In the base case (solid curves), P<sub>NO</sub><sup>rbc</sup> remains constant, and aquaporin-1 (AQP1) is permeable to NO. In the second case (dotted curves), P<sub>NO</sub><sup>rbc</sup> is taken to increase with decreasing P<sub>O2</sub>. In the third case (dash-dotted curves), AQP1 is taken to be impermeable to NO.

Fig. 6. C<sub>O2</sub>– profiles in the interstitium of the 4 concentric regions (R1–R4), assuming a fixed P<sub>NO</sub><sup>rbc</sup>. The solid and dashed curves, respectively, depict C<sub>O2</sub>– with and without O<sub>2</sub>/H<sub>2</sub>O scavenging by NO, assuming that the O<sub>2</sub> generation rate (G<sub>O2</sub>) decreases with decreasing P<sub>O2</sub>. The dotted and dash-dotted curves, respectively, depict C<sub>O2</sub>– with and without O<sub>2</sub> scavenging by NO, assuming that G<sub>O2</sub> is enhanced rather than limited by low medullary P<sub>O2</sub>. C<sub>O2</sub>– increases moderately in the absence of the NO-O<sub>2</sub> reaction because SOD is the main O<sub>2</sub> scavenger.
Specifically, assuming that \( a = 0 \), osmCD drops to 621 mosmol/kgH\(_2\)O under basal conditions (vs. 787 with \( a = 0.5 \)). Abolishing NO-mediated inhibition of mTAL transport has a small impact on NaCl reabsorption, because the supply of O\(_2\) in the interbundle region is not sufficient to support significantly greater metabolic needs by itself. Thus, with \( a = 0 \), osm\(_{L}^{CD}\) only increases by 5% (to 655 mosmol/kgH\(_2\)O) when NO-mediated inhibition of mTAL transport is eliminated. In contrast, in the absence of O\(_2\)-mediated stimulation of NaCl reabsorption, Po\(_2\) reaches comparable levels with and without anaerobic metabolism, NO and O\(_2\) concentration profiles are similar, and so is osm\(_{L}^{CD}\) (464 mosmol/kgH\(_2\)O if \( a = 0 \), vs. 493 if \( a = 0.5 \)).

**AQ1-Mediated NO Transport**

The base case assumes that AQ1 transports NO, as observed experimentally (21). Nonetheless, there is some controversy as to whether AQ1 is indeed permeable to small gases such as CO\(_2\), NH\(_3\), and NO (57). We performed simulations in which AQ1 was taken to be impermeable to NO: the NO permeability of the vessels and tubules that express AQ1 in the OM, namely, DVR and descending limbs, was set to 0.0122 cm/s, equal to that of other vessels and tubules (Table 3). As displayed in Fig. 5, in the absence of NO transport via AQ1, interstitial NO concentrations are predicted to increase significantly relative to the base case. Elevations are most pronounced in the core (R1) and immediate periphery (R2) of the vascular bundle, where all DVR, which represent the largest volumetric source of NO, are located. Interstitial C\(_{NO}\) increases because as the resistance to NO diffusion from endothelium to plasma increases, a smaller fraction of NO makes its way into RBCs, and more NO is preserved elsewhere. The subsequent decrease in mTAL NaCl reabsorption reduces osm\(_{L}^{CD}\) from 787 to 736 mosmol/kgH\(_2\)O (Table 6).

**Flow-Induced Endothelial Nitric Oxide Synthase Activation**

Studies have shown that increased luminal flow activates endothelial nitric oxide synthase (eNOS) and enhances NO production in DVR and TALs (47, 62). Luminal flow also stimulates O\(_2\)-production in TALs (26). Flow-induced effects on O\(_2\) are partly inhibited by NO via a nonscavenging mechanism, as discussed below. To determine whether eNOS and NADPH oxidase activation by luminal flow plays an important role in the regulation of OM Na\(^+\) reabsorption, we performed simulations in which inlet volume flows (i.e., in DVR, descending limbs, and CD at \( x = 0 \)) and maximal NO and O\(_2\) synthesis rates were simultaneously increased by 20%. Vasa recta express both eNOS and neuronal NOS (nNOS) (40), but in the absence of specific data on the distribution of these enzymes, we raised NO (and O\(_2\)) volumetric generation rates by 20% everywhere.

We first examined the isolated effects of increasing inlet volume flows on the OM concentrating mechanism. An increase in medullary perfusion augments O\(_2\) availability and thereby stimulates NaCl reabsorption, but this effect is more than counterbalanced by two opposite forces: a Po\(_2\)-induced increase in NO synthesis, which acts to inhibit NaCl active transport, and higher loads, which mean that larger fluid flows must be concentrated. Thus the OM concentrating capacity is predicted to decrease relative to the base case. As shown in Table 6, an isolated 20% increase in inlet
volume flows is calculated to lower osm \( \overline{C}_{\text{CD}} \) by 18\%, from 787 to 648 mosmol/kgH\(_2\)O.

When inlet volume flows and maximal NO synthesis rates (i.e., \( G_{\text{NO}}^{\text{max}} \) in Eq. 15) are both increased by 20\%, \( C_{\text{NO}} \) rises further, and osm \( \overline{C}_{\text{CD}} \) is predicted to drop even more, to 575 mosmol/kgH\(_2\)O (Table 6). When inlet volume flows, NO synthesis rates, and \( O_2^- \) synthesis rates (i.e., \( G_{\text{O}_2^-/H_2O}^{\text{basal}} \) in Eq. 22a) are all increased by 20\%, \( O_2^- \) exerts greater compensating effects on mTAL transport, and osm \( \overline{C}_{\text{CD}} \) climbs slightly, to 591 mosmol/kgH\(_2\)O (Table 6). Together, these results suggest that an increase in tubular and vascular flows substantially reduces the OM axial osmolality gradient.

![Fig. 8. C\(_{\text{NO}}\) profiles in the interstitium of the 4 regions, in the base case (solid curves), assuming that NO does not inhibit mTAL NaCl reabsorption (dotted curves), and assuming that \( O_2^- \) does not stimulate mTAL NaCl reabsorption (dash-dotted curves). Profiles were obtained assuming that \( P_{\text{NO}}^{\text{rbc}} \) is constant and that \( G_{\text{O}_2^-/H_2O} \) varies in parallel with \( P_{\text{O}_2} \).](http://ajprenal.physiology.org/)

![Fig. 9. C\(_{\text{O}_2^-}\) profiles in the interstitium of the 4 regions, in the base case (solid curves), assuming that NO does not inhibit mTAL NaCl reabsorption (dotted curves), and assuming that \( O_2^- \) does not stimulate mTAL NaCl reabsorption (dash-dotted curves). Profiles were obtained assuming that \( P_{\text{NO}}^{\text{rbc}} \) is constant and that \( G_{\text{O}_2^-} \) varies in parallel with \( P_{\text{O}_2} \).](http://ajprenal.physiology.org/)
Table 6. Effects of luminal flow and O$_2$ synthesis on the concentrating capacity of the OM

<table>
<thead>
<tr>
<th>Condition</th>
<th>osm$^6_{\text{L}}$ mosmol/kgH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Case</td>
<td>787</td>
</tr>
<tr>
<td>Without anaerobic metabolism</td>
<td>621</td>
</tr>
<tr>
<td>Without anaerobic metabolism and no mediated</td>
<td>655</td>
</tr>
<tr>
<td>inhibition of mTAL active transport</td>
<td></td>
</tr>
<tr>
<td>Without anaerobic metabolism and no mediated</td>
<td>464</td>
</tr>
<tr>
<td>stimulation of mTAL active transport</td>
<td></td>
</tr>
<tr>
<td>Without facilitated NO transport via AQPI</td>
<td>736</td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows</td>
<td>648</td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows and in G$_{NO}$</td>
<td>575</td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows, in G$<em>{NO}$ and in G$</em>{O_2}$</td>
<td>591</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$- and a 25% decrease in G$</em>{NO}$</td>
<td>1,073</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$- and a 50% decrease in G$</em>{NO}$</td>
<td>1,175</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$- and a 50% decrease in $T</em>{Na}/Q_{O_2}$</td>
<td>1,297</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$-, a 50% decrease in $T</em>{Na}/Q_{O_2}$, and a 25% decrease in G$_{NO}$</td>
<td>935</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$-, a 50% decrease in $T</em>{Na}/Q_{O_2}$, and a 50% decrease in G$_{NO}$</td>
<td>1,020</td>
</tr>
<tr>
<td>With a 10-fold increase in G$<em>{O_2}$-, a 50% decrease in $T</em>{Na}/Q_{O_2}$, and a 50% decrease in G$_{NO}$</td>
<td>1,124</td>
</tr>
</tbody>
</table>

AQP1, aquaporin-1; G$_{NO}$ and G$_{O_2}$- are the volumetric rates of NO and O$_2$ synthesis, respectively; $T_{Na}/Q_{O_2}$, ratio of transported sodium to oxygen consumption. In all these simulations, G$_{O_2}$- is taken to decrease with decreasing PO$_2$, and $P_{NO}$ is fixed.

Hypertensive Conditions

Medullary infusions of the SOD inhibitor DETC have been shown to induce hypertension in rats: DETC induced an eightfold increase in interstitial C$_{O_2}$-, with a subsequent 40% decrease in medullary blood flow and a nearly 20-mmHg increase in blood pressure (37). To dissect some of the underlying mechanisms, we raised maximal O$_2$ generation rates in the OM by a factor of 10. As expected, the higher levels of O$_2$ stimulate NaCl reabsorption and O$_2$ consumption, thereby reducing PO$_2$ and C$_{NO}$. In the interbundle region, PO$_2$ is predicted to drop by 5–10 mmHg at the mid-OS and 2 mmHg at the mid-IS.

Per se, a 10-fold increase in O$_2$ concentration raises osm$^6_{\text{L}}$ by 300 mosmol/kgH$_2$O, i.e., by 35% (Table 6). The increase is limited because $I$ the effects of O$_2$ on NaCl reabsorption across the mTALs are described using a saturable expression (Eq. 33) and 2) NO still exerts significant inhibitory effects on this NaCl transport pathway. The 35% increase in osm$^6_{\text{L}}$ is predicted assuming that the number of Na$^+$ moles actively reabsorbed per mole of O$_2$ consumed (i.e., the mTAL $T_{Na}/Q_{O_2}$ ratio) remains fixed at 18. There is some evidence, however, that NO and/or reactive oxygen species (ROS) modulate the amount of O$_2$ consumed per Na$^+$ ion transported, as discussed below. However, the explicit impact of NO and ROS on $T_{Na}/Q_{O_2}$ in the mTAL has not been measured, to the best of our knowledge. If we assume that this ratio is halved (based on data from spontaneously hypertensive rats in Ref. 59) when O$_2$ concentrations increase 10-fold, our model then predicts a smaller (20%) increase in osm$^6_{\text{L}}$ (Table 6).

Some models of hypertension, such as the Dahl salt-sensitive rat, are also characterized by a decrease in NO production (39). In the absence of specific data, we simulated graded reductions (25 and 50%) in NO synthesis in parallel with the 10-fold increase in O$_2$ levels. As expected, the greater the reduction in G$_{NO}$, the faster NaCl transport across mTALS, and the higher the concentrating capacity of the OM (Table 6). The impact of the G$_{O_2}$- increase, G$_{NO}$ decrease, and $T_{Na}/Q_{O_2}$ variations on NaCl reabsorption along the long ascending limb (LAL) is illustrated in Fig. 10. The Na$^+$ flow at the LAL inlet (at $x = L$) is very similar in all cases, −27 pmol/s (per tubule). In the base case, it decreases to 5.8 pmol/s at the corticomедullary junction (x = 0). If the O$_2$ synthesis rate is multiplied by 10 and Na$^+$ reabsorption thereby stimulated, it decreases much more rapidly. If $T_{Na}/Q_{O_2}$ remains equal to 18, the LAL Na$^+$ flow decreases to 1.6 and 0.5 pmol/s at $x = 0$, assuming no change and a 50% decrease in G$_{NO}$, respectively. If $T_{Na}/Q_{O_2}$ drops to 9, it decreases slightly less, to 3.0 and 1.3 pmol/s, respectively.

DISCUSSION

**Feedback Mechanisms**

Our results suggest that NaCl reabsorption across mTALs and the concentrating capacity of the OM are substantially modulated by NO and O$_2$. Moreover, the effect of each solute on NaCl transport in the OM cannot be considered in isolation, given the feedback loops resulting from the reciprocal interactions between O$_2$, NO, and O$_2$ -, which are summarized in Fig. 11.

The net production rate of NO and O$_2$ is oxygen dependent. Reciprocally, NO and O$_2$ affect oxygen availability in two ways: by modulating active transport across mTAL cells and therefore O$_2$ consumption, and by regulating vessel contraction and therefore O$_2$ supply. Our current model does not take into account the effect of NO on vessel contraction.
Nitric oxide and superoxide modulate NaCl transport and PO2

Fig. 11. Feedback mechanisms involving NO, O2, and O2. NO synthesis is O2 dependent, but RBC trapping of NO may also decrease under hypoxic conditions, via uncertain mechanisms. NO is scavenged very rapidly by hemoglobin (Hb), and to a smaller extent by O2. The relationship between O2 synthesis and PO2 remains unclear. Superoxide dismutase (SOD) consumes O2 at a faster rate than NO does. As a vasodilator, NO enhances medullary perfusion and O2 supply, whereas O2 exerts opposite effects. NO inhibits NaCl reabsorption by the thick ascending limb and therefore O2 consumption, whereas O2 stimulates both. Not shown on the diagram are the many other paracrine agents (such as endothelins and prostaglandins) that also act on vascular contraction and tubular NaCl reabsorption in the renal medulla.

Our prediction that active transport of NaCl across mTALs, faster transport rates and therefore enhanced O2 consumption would reduce PO2, which in turn would lower NO production. The resulting decrease in CNO should then lead to vasoconstriction, which would further reduce PO2 by limiting O2 supply. However, several mechanisms would then put a break on this positive feedback loop. Hypoxic-induced NO release should partly counteract the reduction in NOS-mediated NO synthesis, as discussed above. Moreover, the medullary microcirculation is controlled by many signaling molecules. In particular, adenosine and prostaglandins, like NO, act both as saluretic agents and as paracrine vasodilators; such agents would most likely exert mitigating effects so as to preserve medullary perfusion and raise medullary PO2. Similarly, activation of KATP channels due to reduction of intracellular ATP might hyperpolarize vasa recta pericytes to favor vasodilatation (5).

Conversely, without O2-mediated stimulation of sodium reabsorption and O2 consumption, elevation of PO2 would favor a rise in CNO and vasodilation, tending to enhance O2 supply. In this case, however, active transport by the mTAL would also tend to increase, since it is partly limited by O2 availability under basal conditions. Thus a rise in mTAL O2 consumption would be favored, tending to offset the increase in O2 supply. Finally, it seems plausible that other paracrine agents such as endothelins or vasoconstrictor prostaglandins might be released to limit such NO-dependent vasodilatation. Given the complex nature of events that balance O2 supply and consumption, we formulated the current model to facilitate prediction of the net effect of such interactions.

Impact of NO on O2 Bioavailability

Our model predicts that NO scavenging reduces O2 levels by ~10% in the renal medulla. As recently observed by Hong and Garvin (25), flow-induced enhancement of O2 production in the TAL is reduced in the presence of NO, but this effect cannot be attributed to scavenging only. Approximately 70% of the inhibitory effect of NO on net O2 production appears to be mediated via the cGMP/PKG pathway (25). These novel findings suggest that NO and O2 may interact in more complex ways than previously thought. It is not presently known under which conditions, and precisely how, the cGMP/PKG pathway leads to inhibition of net O2 production. In the absence of data, we did not incorporate this pathway in our model.

Impact of O2 on NO Bioavailability

The impact of basal O2 levels on NO bioavailability in the OM remains to be fully ascertained, in part because there have been until now no direct measurements of medullary O2 levels. In a recent mathematical model of NO-O2 interactions in OM cross sections (17), we showed that if O2 is present in subnanomolar concentrations, it affects NO to a small extent only. In the current study, we assumed that O2 is present at higher levels (1–10 nM), based on measured H2O2 concentrations. Thus CNO increased significantly when the NO-O2 reaction rate was set to zero (Fig. 7). Moreover, when the effects of O2 on mTAL reabsorption were abolished, CNO increased even more in the interbundle region (Fig. 8). In other words, the present model indicates that O2 modulates NO levels both directly and indirectly: O2 may indirectly reduce NO generation via its stimulation of mTAL metabolic requirements, leading to reduction of O2 as a substrate for NO formation in the OM. It is likely, however, that hypoxia-induced NO release acts to compensate for the decrease in NO synthesis. Our current model suggests that hypoxia-mediated effects would not suffice to fully counteract the GNO decrease, but complete elucidation of the mechanisms by which hypoxia raises NO levels is needed to draw definitive conclusions.

Experimental observations regarding the effects of basal O2 on NO bioavailability are conflicting. Cowley and colleagues (11) observed that renal medullary interstitial infusion of the SOD mimetic tempol in anesthetized rats raised medullary blood flow and sodium excretion; these effects were partly counteracted by the dismutation of superoxide into H2O2, but were not affected by pretreatment with a NOS inhibitor (63). These results, combined with the observation that tempol by itself does not affect the basal tone of microperfused descending vasa recta (4), suggest that basal O2 has little direct impact on medullary NO levels. In contrast, several ex vivo studies
have found that tempol enhances the release and diffusion of NO from mTALs (41, 45). However, these studies were performed in the absence of RBCs (i.e., of hemoglobin), and with a disrupted vascular endothelium (which, particularly when stimulated by shear, constitutes the main source of NO in vivo); under such conditions, $O_2^-$ would have had a disproportionate impact on NO levels.

In contrast, studies consistently indicate that $O_2^-$ significantly reduces NO bioavailability under oxidative stress conditions. In spontaneously hypertensive rats, or in rats infused with ANG II, tempol markedly affects arterial blood pressure, renal blood flow, glomerular filtration rate, and/or sodium excretion (28, 44, 54). Pretreatment with $N^\bullet$-nitro-$l$-arginine methyl ester ($l$-NAME) eliminates the antihypertensive effects of tempol (53), implying that, in these hypertensive animals, $O_2^-$ is present at sufficient levels to significantly scavenge NO.

**Hypertensive Conditions**

Our model predicts that shifting the NO-.$O_2^-$ balance in favor of superoxide substantially enhances NaCl reabsorption across mTALs (Fig. 10) and further depletes oxygen in the OM, as experiments have suggested. Our simulations suggest that a 10-fold increase in the rate of $O_2^-$ synthesis raises the concentrating capacity of the OM by $\sim$35%, and reduces $P_{O_2}$ in the peripheral regions by 5–10 mmHg in the OS and 1–3 mmHg in the IS, assuming that the mTAL $T_{Na}/Q_{O_2}$ ratio remains constant. In fact, the number of Na$^+$ moles reabsorbed per mole of $O_2$ consumed is reported to be lower in hypertensive subjects than controls (26), but also induces NOS activation in DVR and TALs. Shear production, via PKC-mediated activation of NADPH oxidase (47). We previously showed that per se, increases in vascular and tubular flows lower the OM concentrating capacity: despite greater $O_2$ availability, the higher loads make it harder to concentrate the tubular fluid (7). Our earlier study, which did not include the transport of NO and $O_2^-$, predicted that a 25% increase in volume flows lowers osm$^CD_{CD}$ by 10%. The current model suggests that increasing volume flows may in fact reduce the osmolarity gradient even more, because NO-mediated inhibition of mTAL transport intensifies. The flow-induced increase in $O_2^-$ synthesis, if comparable to that in NO synthesis, is not sufficient to offset these effects. Hence, in the absence of other counteracting mechanisms, a 20% increase in flow rates that is accompanied by a 25% increase in the production of NO and $O_2^-$ is predicted to lower osm$^CD_{CD}$ by $\sim$25% (Table 6).

**Model Validation, Comparison, and Limitations**

There are very few experimental data with which our model predictions can be compared and validated. Predicted NO concentrations in the OM fall within the range of measured values, which extend from 60–100 nM (27, 64) to 800 nM (52), but there have been no direct measurements of $O_2$ levels in the OM, to the best of our knowledge. We compared the predicted and measured reduction in $P_{O_2}$ induced by the NOS inhibitor $l$-NAME. Li et al. (33) used BOLD MRI to detect changes in renal medullary oxygenation. In Wistar-Kyoto rats, the parameter R2* (which is inversely proportional to $P_{O_2}$) increased by $\sim$40% following administration of $l$-NAME. Similarly, we found that abolishing NO generation reduced interstitial $P_{O_2}$ in the interbundle region by 30–50%, depending on position (results not shown).

The current model differs from our previous model of NO and $O_2^-$ transport in the OM (16, 17) in several important respects. The latter was restricted to medullary cross sections, focused on the transport of three solutes only (NO, $O_2^-$, and ONOO$^-$), and assumed fixed $P_{O_2}$ profiles. In particular, it did not consider the effects of NO and $O_2^-$ on sodium reabsorption and $O_2$ consumption in the OM, and therefore failed to capture important reciprocal interactions. In addition, basal $O_2$ concentrations were previously taken to be subnanomolar, based on measured $O_2^-$ synthesis rates in aortic endothelial cells. In this study, basal $O_2$ concentrations are taken to be $\sim$10 times higher, based on experimental determinations of medullary $H_2O_2$.

Some of the significant limitations of our study were discussed above. In the absence of quantitative data, several model parameters are necessarily uncertain, particularly those related to NO- and $O_2^-$-mediated effects on NaCl reabsorption in the mTAL. In addition, our steady-state model does not account for vasomotion and hormone-induced changes in vessel diameter. Finally, we did not examine the effects of ONOO$^-$ (the product of the NO-$O_2^-$ reaction) and $H_2O_2$ (the product of the SOD-$O_2^-$ reaction) on medullary function. ONOO$^-$ is thought to exert potent cytotoxic effects at high concentrations and to induce vascular relaxation at lower (nM) concentrations, but the role of ONOO$^-$ in regulating kidney function remains to be investigated (36). Similarly, the far-reaching vascular effects of $H_2O_2$ have yet to be fully characterized in vivo (1).

In vivo, solute reabsorption and medullary blood flow are controlled by many endocrine and paracrine factors. This study aimed to yield a better understanding of the interactions between NO and $O_2^-$ and their combined effects on tubular and vascular function in the OM. Given the complex nature of events that balance $O_2$ supply and consumption and modulate NaCl transport, we formulated the current model to facilitate prediction of the net effect of such interactions.
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