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 conditions. Our results suggest that NaCl transport and the concentration of oxygen are raised indirectly via its stimulation of mTAL metabolism, leading to a 40% lower. Conversely, without NO-induced inhibition of NaCl active transport, the outer medullary concentrating capacity would increase by ~70%. 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 ~10-fold, as in hypertensive animals, mTAL NaCl reabsorption is reduced by 10-fold, as in hypertensive animals, mTAL NaCl reabsorption is significantly enhanced, even as the inefficient use of O2 exacerbates hypoxia in the outer medulla. Conversely, an increase in tubular and vascular flows is predicted to substantially reduce mTAL NaCl reabsorption. In conclusion, our model suggests that the complex interactions between NO, O2, and O2 significantly impact the O2 balance and NaCl reabsorption in the outer medulla.

concentrating ability; kidney; oxygen balance; reactive oxygen species; sodium reabsorption

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. 1B 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
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).

This section focuses on the new features of our model: the transmural flux of water and solute \(k\) into tubule \(i\); \(A_{\text{lum}}^k\) and \(A_{\text{epi}}^k\), respectively, designate the cross-sectional area of the lumen of \(i\) (i.e., based on its inner diameter) and that of the surrounding epithelium. \(\Omega_{i,k}^{\text{lum}}\) and \(\Omega_{i,k}^{\text{epi}}\) are the volumetric consumption rate of solute \(k\) in the lumen of tubule \(i\) and that of the surrounding epithelium, and \(G_{i,k}^{\text{epi}}\) 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. \(F_{i, V}^{\text{pl}}\) and \(F_{i, V}^{\text{rbc}}\) denote the plasma and RBC water flow rate in vessel \(i\), respectively, so that the total water flow rate in vessel \(i\) is \(F_{i, V} = F_{i, V}^{\text{pl}} + F_{i, V}^{\text{rbc}}\). Similarly, \(C_{i, V}^{\text{pl}}\) and \(C_{i, V}^{\text{rbc}}\) 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

\[
\frac{\partial F_{i, V}^{\text{pl}}(x)}{\partial x} = j_{i, V}^{\text{pl}}(x) \\
\frac{\partial F_{i, V}^{\text{rbc}}(x)}{\partial x} = j_{i, V}^{\text{rbc}}(x)
\]

where \(j_{i, V}^{\text{pl}}\) is the net transmural flux of water into plasma (i.e., from the interstitium and RBCs) and \(j_{i, V}^{\text{rbc}}\) that into RBCs. Note that \(j_{i, V}^{\text{pl}} = j_{i, V}^{\text{pl, int}} - j_{i, V}^{\text{pl, rbc}}\), where \(j_{i, V}^{\text{pl, int}}\) designates the transmural flux from interstitium to plasma.

Similarly, solute conservation in the plasma and RBC compartments of blood vessel \(i\) is expressed as
\[ \frac{\partial F_i^{\text{PL}}(x)C_i^{\text{PL}}(x)}{\partial x} = J_i^{\text{PL}}(x) + 0A_i^{\text{endo}}(x)\left[ G_i^{\text{endo}}(x) - \Omega_i^{\text{endo}}(x) \right] - A_i^{\text{PL}}(x)\Omega_i^{\text{PL}}(x) \]  
\[ \frac{\partial F_i^{\text{RC}}(x)C_i^{\text{RC}}(x)}{\partial x} = J_i^{\text{RC}}(x) - A_i^{\text{RC}}(x)\Omega_i^{\text{RC}}(x) \]  

where \( J_i^{\text{PL}} \) and \( J_i^{\text{RC}} \) are the net transmural flux of solute \( k \) entering plasma and RBCs; \( \Omega_i^{\text{PL}} \) and \( \Omega_i^{\text{RC}} \) represent the fraction of net solute produced by tubular epithelial and short ascending vasa recta into \( R \). The third and fourth term denotes the consumption rate of solute \( k \) by interstitial cells in region \( R \). For example, the net consumption rate of solute \( k \) by interstitial cells in region \( R \) yields the interstitial concentration of solute \( k \) at any location within region \( R \). 

\[ J_{i,k} = 2\pi r_i \sum_{\text{region } R} \kappa_{i,k} \left[ P_{i,k}(C_{i,k} - C_{R,k}) - \Psi_{i,k}^{\text{active}} \right] \]  

where \( r_i \) is the inner radius of the tube, \( d_i \) is the product of the partial molar volume of water and the osmotic water permeability of \( i \), \( \kappa_{i,k} \) is the reflection coefficient for solute \( k \) (taken to be 1 for all solutes), \( \Phi_0 \) is the osmotic coefficient for solute \( k \), and \( C_{R,k} \) is the concentration of solute \( k \) in region \( R \). \( P_{i,k} \) denotes the permeability of solute \( k \) to region \( R \), and \( \Psi_{i,k}^{\text{active}} \) the rate of active transport of solute \( k \). As illustrated in Fig. 1, in some cases, fractions of a tubule or vas rectum \( \alpha \) are distributed between two concentric regions, and \( \kappa_{i,k} \) is the fraction of that is in contact with region \( R \). The spatial dependence of the variables in the flux equations has been omitted for simplicity. 

**Vasa recta.** The flux of water flowing from the interstitium into DVR plasma is given by 

\[ J_i^{\text{int}} = 2\pi r_i d_i \sum_{\text{region } R} \left[ \kappa_{i,R} \frac{\sum_k \sigma_k \Phi_k(C_{i,k}^{\text{PL}} - C_{i,k}^{\text{RC}})}{\sum_k \sigma_k (C_{i,k}^{\text{PL}} - C_{i,k}^{\text{RC}})} \right] \]  

The flux of water from plasma into RBCs is expressed as 

\[ J_i^{\text{pl}} = 2\pi r_i d_i \sum_{\text{region } R} \left[ \frac{1}{N_R T} \frac{\Delta \pi_R - \Delta \pi_p}{\frac{A_{\text{PL}}}{V_i}} + \frac{\sigma_k \Phi_k(C_{i,k}^{\text{RC}} - C_{i,k}^{\text{PL}})}{\sum_k \sigma_k (C_{i,k}^{\text{RC}} - C_{i,k}^{\text{PL}})} \right] \]  

The net transmural flux of solute \( k \) flowing from the interstitium into DVR plasma, and that from plasma to RBCs, are given by 

\[ J_{i,k}^{\text{int}} = 2\pi r_i d_i \sum_{\text{region } R} \left[ \kappa_{i,R} \frac{\sum_k \sigma_k \Phi_k(C_{i,k}^{\text{PL}} - C_{i,k}^{\text{RC}})}{\sum_k \sigma_k (C_{i,k}^{\text{PL}} - C_{i,k}^{\text{RC}})} \right] \]  

\[ J_{i,k}^{\text{pl}} = 2\pi r_i d_i J_{i,k}^{\text{RC}}(C_{i,k}^{\text{PL}} - C_{i,k}^{\text{RC}}) \]  

Note that \( J_{i,k}^{\text{PL}} = J_{i,k}^{\text{int}} - J_{i,k}^{\text{RC}} \). 

**NO Generation and Consumption.** 

NO is generated in the vascular endothelium and tubular epithelium, and its synthesis rate depends on \( O_2 \) availability. The \( O_2 \) dependence of \( G_i \), NO is modeled using a Michaelis-Menten relationship 

\[ G_{\text{NO}}^{\text{cell}}(x) = G_{\text{NO}}^{\text{max}} \left[ \frac{C_{\text{NO}}(x)}{C_{\text{O}_2}(x)} \right] \text{cell = endothelium, epithelium} \]  

where the Michaelis-Menten constant \( K_{\text{NO}}^{\text{O}_2} \) (taken as 38 mmHg, based on Ref. 60) is the oxygen concentration at half the maximal rate of NO production in cell layer \( i \), itself denoted by \( G_{\text{NO}}^{\text{max}} \), NO is the total fluid accumulation carried away by AVR, and \( A_{\text{PL}} \) 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 \( R' \). 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 term of square brackets represents the solute flux from SDV terminating at level \( y = x \) into region \( R \). The next term in that first term of square brackets is the solute flux from capillary RBCs into \( R \). The term \( C_{\text{R},k} \), \( \omega_{\text{R},k} = C_{\text{AVR}} + \Omega_{\text{R},k} \) represents the net amount of solute that is carried by flow at the local concentration into AVR or into an adjoining region \( R' \). 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 first-to-last term denotes the net amount of solute \( k \) produced by capillary endothelium in region \( R \) (see below), and the last term denotes the consumption rate of solute \( k \) by interstitial cells in region \( R \).
NITRIC OXIDE AND SUPEROXIDE MODULATE NaCl TRANSPORT AND PO2

\[ \Omega_{NO} = V_{i,1} + V_{i,2} + V_{i,3} + V_{i,4} \]  \hspace{1cm} (20)

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.

Hyoxia-Induced NO Release

Even though oxygen is a precursor in the synthesis of NO, hypoxia has been shown to raise medullary NO levels in anesthetized rats (22). Several mechanisms have been proposed to explain how hypoxia may induce NO release. Stamler and colleagues (50) have postulated that RBC nitrite is reduced by deoxyhemoglobin to form NO, and low PO2 stimulates NO release via this route (14). Some of the key steps in these processes remain to be fully elucidated. In a previous model (16), we used the approach developed by Chen et al. (9, 10) to model the SNOHb and RBC nitrite pathways, which suggested that neither significantly affects medullary NO concentrations. Thus, in the current model, we account for hypoxia-induced stimulation of NO release in the OM in a simple manner: we assume that as O2 availability decreases, the RBC permeability to NO (\( P_{NO}^{bc} \)) also decreases, thereby preserving more NO in kidney tissue. Specifically, \( P_{NO}^{bc} \) is taken to vary linearly with PO2 according to

\[ P_{NO}^{bc} = P_{NO}^{bc,basal} \left( \frac{1}{2} \cdot \frac{\text{PO}_2 - P_a}{\text{PO}_2 - P_a} \right) \]  \hspace{1cm} (21)

where the basal RBC permeability to NO (\( P_{NO}^{bc,basal} \)) is taken as 0.1 cm/s (16). The parameters of Eq. 21 are chosen so that our previous model of NO transport (16) predicts a 14% increase in medullary NO levels (at the mid-IS in R3–R4) when medullary PO2 decreases from \( P_a = 28 \) mmHg to \( P_a = 12 \) mmHg, as observed experimentally when indomethacin is administered to anesthetized rats (23).

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 production (34), whereas others report an inhibitory effect (12), we consider two different hypotheses. Case A assumes that low PO2 inhibits O2 synthesis, and the oxygen dependence of the O2 generation rate is then modeled using a Michaelis-Menten relationship

\[ G_{i,2}(x) = G_{basal}^{i,2} \left( \frac{C_{i,2}(x)}{K_{O2}^{i,2} + C_{i,03}(x)} \right) \]  \hspace{1cm} (22a)

where \( G_{basal}^{i,2} \) is fixed, and \( K_{O2}^{i,2} \) is taken as 15.4 mmHg (12). Case B assumes that low PO2 increases O2 production by 50% relative to well-oxygenated conditions (34), so that the rate of O2 synthesis under basal conditions is given by

\[ G_{i,2}(x) = 1.5 \cdot G_{basal}^{i,2} \]  \hspace{1cm} (22b)

The \( G_{basal}^{i,2} \) values between vasa recta and the different types of tubules are based upon experimental measurements (34), and the basal rate of O2 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 \( \Omega_{i} \) of the latter reaction is calculated as

\[ V_{i,5} = k_{SO2} C_{i,02} - C_{i,SOD} \]  \hspace{1cm} (23)

The total volumetric consumption rate of O2 in compartment \( i \) is given by

\[ \Omega_{i,2} = V_{i,2} + \Theta_{i,5} \]  \hspace{1cm} (24)

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

\[ 0 = \frac{d[H_2O_2]}{dt} = k_{SO2} C_{i,02} - k_{cat} \left[ C_{i,SOD} \right] [H_2O_2] \]  \hspace{1cm} (25)

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-catalase catalysis activity ratio of 0.4 (56), we estimate the renal concentration of catalase to be on the order of 5 µM. Assuming that \( J \) the intracellular concentration of SOD is 10 µM (18), \( k_{cat} \) equals \( 3.4 \times 10^7 \text{ M}^{-1} \text{s}^{-1} \) (42), and \( k_{SO2} \) equals \( 1.6 \times 10^7 \text{ M}^{-1} \text{s}^{-1} \) (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. 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

\[ A_R^{cap,endo}(x) = n_{cap} \cdot (-d_{SDV}/dx) \cdot \pi \cdot [(r_{cap} + d_{endo})^2 - (r_{cap} - r_{R-1})^2 \cdot (r_{R-1} - r_K) \cdot (r_K - r_{R-1})] \]  \hspace{1cm} (26)

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

\[ \Omega_{i,RBC} = -V_{i,4} \]  \hspace{1cm} (27)

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 (\(\Omega_{mTAL,O2}^{active}\)) is calculated as

\[
\Omega_{mTAL,O2}^{active}(x) = \frac{2 \pi r_{mTAL}(x) \Psi_{mTAL,Na}^{active}(x) \Theta(P_{mTAL,O2})}{18A_{mTAL}^{int}(x)}
\]

(28)

where \(\Psi_{mTAL,Na}^{active}\) denotes the mTAL active Na\textsuperscript{+} transport rate, and \(\Theta(P_{mTAL,O2})\) is the fraction of that transport rate that is supported by aerobic respiration, given by

\[
\Theta(P_{mTAL,O2}) = \begin{cases} 
1 & P_{mTAL,O2} \geq P_c \\
\frac{P_{mTAL,O2}/P_c}{a + (1 - a)(P_{mTAL,O2}/P_c)} & P_{mTAL,O2} < P_c
\end{cases}
\]

(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

\[
\Omega_{basal,i}(x) = \frac{Q_{basal}^{max,i} C_{O2}(x)}{K_{M,i}(x) + C_{O2}(x)}
\]

(30)

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

\[
K_{M,i}^{NO}(x) = K_{M,i}^{NO,basal} \left[ 1 + C_{i(NO)}(x)/C_{NO}^{inhb} \right]
\]

(31)

where \(K_{M,i}^{NO,basal}\) is the Michaelis-Menten constant in the absence of NO, and \(C_{NO}^{inhb}\) is the NO concentration that doubles \(K_{M,i}\); 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

\[
\Psi_{mTAL,Na}^{active}(x) = \frac{V_{max,Na}(x) C_{mTAL,Na}(x)}{K_{M,Na} + C_{mTAL,Na}(x)}
\]

(32)

where \(V_{max,Na}\) (in mol Na\textsuperscript{+}·m\textsuperscript{-2}·s\textsuperscript{-1}) is the maximal rate of Na\textsuperscript{+} transport, and \(K_{M,Na}\) 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 \(\Psi_{mTAL,Na}^{active}\) are incorporated separately as follows

\[
V_{max,Na} = V_{max,Na}^{0} \cdot f(C_{mTAL,O2}) \cdot g(C_{mTAL,NO}) \cdot h(C_{mTAL,O2})
\]

(33)

where \(V_{max,Na}^{0}\) is a constant. We assume that below P\textsubscript{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

\[
f(C_{mTAL,O2}) = \begin{cases} 
1 & P_{mTAL,O2} \geq P_c \\
(1 - a)C_{mTAL,O2}/(\alpha_{O2}P_c) & P_{mTAL,O2} < P_c
\end{cases}
\]

(34)

where \(\alpha_{O2}\) is the O\textsubscript{2} solubility coefficient, taken as 1.34 \(\mu\)M/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 \(\Psi_{mTAL,Na}^{active}\) we assume that \(V_{max,Na}\) decreases with increasing NO concentration according to

\[
g(C_{mTAL,NO}) = 1 - \frac{C_{mTAL,NO}}{\beta + C_{mTAL,NO}}
\]

(35)

Ortiz et al. (48) reported that 10 \(\mu\)M spermine NONOate (or SPM, an NO donor) inhibits mTAL Cl\textsuperscript{-} reabsorption by 46%. At a concentration of 10 \(\mu\)M, 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 \(\mu\)M) 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{3} 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 (\(C_{mTAL,O2}^{0}\)) to zero decreases NaCl active transport by 30%. Specifically, we assume that \(V_{max,Na}\) increases with increasing O\textsubscript{2} concentration according to

\[
h(C_{mTAL,O2}) = 0.7 + 0.6 \left( \frac{C_{mTAL,O2} - C_{mTAL,O2}^{0}}{C_{mTAL,O2} - C_{mTAL,O2}^{0} + C_{mTAL,O2}^{0}} \right)
\]

(36)

The reference values (\(C_{mTAL,O2}^{0}\)) are chosen so that \(h = 1\) in the basal configuration. Based upon preliminary simulations, \(C_{mTAL,O2}^{0}\) is taken as 20 pM in case A and 350 pM in case B. The constant \(V_{mTAL,Na}\) was previously estimated as 25.9 mmol/(cm\textsuperscript{2}·s) in the inner stripe, and 10.5 in the outer stripe, in a model that did not account for 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 \(V_{mTAL,Na}\) values are multiplied by 2.2.

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

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

\[
P_{i,NO} = \frac{P_{b,basal}}{60.64P_{i} + 20.23}
\]

(37)

where \(P_{b}\) 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) \(P_{b}\) 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 \(5 \times 10^{-4}\) cm/s.

The effective permeability to solute \(k = (NO, O_2)\) 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 linearly along the descending vasa recta. Thus the concentration of solute long ascending and descending limbs, and in long ascending and 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 HbNO leaving LDV is taken to be equal to that entering LAV

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

The RBC concentration of HbNO is specified as 1 μM in descending vasa recta at the corticomedullary junction. The factor \( \gamma \) equals 5 for both NO and 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 O\(_2\) in dilute solution is taken as 3,300 and 2,800 \( \mu \)m/s, respectively (6, 38).

**Boundary Conditions**

The boundary conditions for the flows of water, Na\(^+\), urea, O\(_2\), Hb, and HbO\(_2\) were described previously (8). The concentrations of NO and O\(_2\) are specified at the corticomedullary junction in descending vessels and tubules (Table 1). They must also be prescribed in long ascending limbs (LAL) and long ascending vasa recta (LAV) at the boundary between the inner and outer medulla (i.e., at \( x = L \)). To do so, we assume that NO and O\(_2\) molar flow rates at \( x = L \) are equal in long ascending and descending limbs, and in long ascending and descending vasa recta. Thus the concentration of solute \( k = NO \) and O\(_2\) in LAL fluid and LAV plasma at \( x = L \) is obtained by solving the following equations.

**Long ascending limbs.**

\[
n_{LAL} F_{LAL}(L) C_{LAL}(L) = n_{LDB} F_{LDB}(L) C_{LDB}(L) \quad (39)
\]

**Long vasa recta.**

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

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

We also assume that the RBC-to-plasma concentration ratio for solute \( k = NO \) and O\(_2\) is the same in long ascending and descending vasa recta at \( x = L \).
Table 4. Reaction kinetic parameters

<table>
<thead>
<tr>
<th>Parameter Definition</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO-O2 reaction rate constant $k_{O2}$, M$^{-2}$s$^{-1}$</td>
<td>$6.3 \times 10^6$</td>
</tr>
<tr>
<td>NO-O2 reaction rate constant $k_{NO,O2}$, M$^{-1}$s$^{-1}$</td>
<td>$6.7 \times 10^6$</td>
</tr>
<tr>
<td>NO-HbO2 reaction rate constant $k_{HbO2}$, M$^{-1}$s$^{-1}$</td>
<td>$2.5 \times 10^7$</td>
</tr>
<tr>
<td>NO-HbO2 reaction rate constant $k_{HbO2}$, M$^{-1}$s$^{-1}$ and $k_{O2}$, s$^{-1}$</td>
<td>$2.5 \times 10^7$ and $10^{-4}$</td>
</tr>
<tr>
<td>O$<em>2$-SOD reaction rate constant $k</em>{O2,SOD}$, M$^{-1}$s$^{-1}$</td>
<td>$1.6 \times 10^6$</td>
</tr>
<tr>
<td>SOD concentration, µM</td>
<td>5</td>
</tr>
</tbody>
</table>

HbO$_2$, oxyhemoglobin.
to \(O_2^-\), and vascular and tubular permeabilities to \(O_2^-\) are taken to be significantly lower than those to NO. Thus transmembrane \(C_{O2^-}\) gradients are predicted to be significant, and in a given region, \(C_{O2^-}\) is significantly higher in the interstitium than in tubular lumen or plasma. In the base case, the \(O_2^-\) generation rate is assumed to decrease with decreasing \(P_{O2}\), and close examination of the curves reveals that interstitial \(C_{O2^-}\) profiles closely track \(P_{O2}\) profiles. Note that \(C_{O2^-}\) is more elevated in R1 than in the peripheral regions not only because \(P_{O2}\) 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 \(C_{O2^-}\) 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 osmality of the tubular fluid in the CD at the OM-IM junction (denoted \(osm_{CD}\) hereafter). Under basal conditions, the latter equals 787 mosmol/kgH2O (Table 5).

**Effects of Hypoxia**

As described above, the mechanisms by which hypoxia modulates medullary NO and \(O_2^-\) levels remain uncertain. To incorporate the effects of hypoxia on \(C_{NO}\) in a simple manner, we assumed that the RBC permeability to NO (\(P_{NO}^{\text{rbc}}\)) is kept constant, and the \(O_2^-\) generation rate (\(G_{O2^-}\)) is taken to vary in parallel with \(P_{O2}\).

Fig. 2. Oxygen tension (\(P_{O2}\)) 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 \(O_2^-\) 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 (\(P_{NO}^{\text{rbc}}\)) is kept constant, and the \(O_2^-\) generation rate (\(G_{O2^-}\)) is taken to vary in parallel with \(P_{O2}\).

The extent to which \(C_{NO}\) is modulated by the NO–\(O_2^-\) reaction depends on \(O_2^-\) levels, as displayed in Fig. 5. Higher \(O_2^-\) levels in mTALs result in greater inhibition of \(Na^+\) transport, and thus a diminished concentrating capacity; \(osm_{CD}\) is predicted to be 729 mosmol/kgH2O in this case.

As to the effects of hypoxia on \(O_2^+\), if we assume that a low \(P_{O2}\) enhances rather than limits \(O_2^-\) generation (as described by Eq. 22b), the predicted \(C_{O2^-}\) is then two to three times higher than in the base case (Fig. 6). Since the volumetric generation rate of \(O_2^-\) is highest in vasa recta and descending limbs, interstitial \(C_{O2^-}\) varies in proportion to the fractional area occupied by vasa recta and descending limbs within each region, as previously described (16). In the OS, this fractional area is highest in R1 and lowest in R2, and so is \(C_{O2^-}\). In the deep IS, the relative area occupied by vasa recta and descending limbs decreases monotonically from R1 to R4, and so does interstitial \(C_{O2^-}\). The CD fluid osmolality at the OM-IM junction is predicted to be 878 mosmol/kgH2O in this case.

**Direct NO–\(O_2^-\) Interactions**

To assess the impact of direct NO–\(O_2^-\) interactions, we then set the \(NO-O_2^-\) reaction rate to zero. Simulations were performed for three cases: \(G_{O2^-}\) and \(P_{NO}^{\text{rbc}}\) were taken to be fixed or to vary with \(P_{O2}\). In all three scenarios, predicted \(O_2^-\) concentrations rose by 10% at most (Fig. 6), because the rate of \(O_2^-\) consumption by SOD is significantly faster than that by NO.

The extent to which \(C_{NO}\) is modulated by the NO–\(O_2^-\) reaction depends on \(O_2^-\) levels, as displayed in Fig. 7. If \(G_{O2^-}\) and \(C_{O2^-}\) are low to start with (as in the base case), eliminating the NO–\(O_2^-\) reaction raises \(C_{NO}\) by 2–10 nM, and \(osm_{CD}\) drops slightly, from 787 to 770 mosmol/kgH2O (Table 5). If \(C_{O2^-}\) is two to three times higher (as when \(G_{O2^-}\) is enhanced by low medullary \(P_{O2}\)), eliminating the NO–\(O_2^-\) reaction raises \(C_{NO}\) by up to 30 nM in R3–R4, i.e., those regions where the major NO scavenger, hemoglobin, is the least predominant. In that case, \(osm_{CD}\) is predicted to decrease by 20%, from 878 to 714 mosmol/kgH2O (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 \(g(C_{\text{mTALNO}}) = 1\) in Eq. 33. In the absence of NO-mediated inhibition, the rates of NaCl reabsorption and \(O_2^-\) consumption are predicted to both rise markedly relative to the
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 OM (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.

**Fig. 3. Base case NO concentration (CNO) in tubules, vasa recta, and interstitia. A–D: regions R1, R2, R3, and R4, respectively; tubules are assigned to the region with which they are in contact for 50% or more of their inner stripe length. E: CNO profiles in the interstitium of the 4 regions.**
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\textsuperscript{-} on the concentrating capacity of the OM

<table>
<thead>
<tr>
<th>Case</th>
<th>Base case</th>
<th>Without the NO/O2\textsuperscript{-} reaction</th>
<th>Without NO-mediated inhibition of mTAL active transport</th>
<th>Without O2\textsuperscript{-}-mediated stimulation of mTAL active transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>osmCD\textsubscript{L}, mosmol/kgH2O</td>
<td>787</td>
<td>770</td>
<td>1,333</td>
<td>493</td>
</tr>
</tbody>
</table>

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

Without O2\textsuperscript{-}-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\textsuperscript{-} effects on NaCl transport are eliminated, the PO2 elevation translates into an increase in O2\textsuperscript{-} generation, assuming that O2\textsuperscript{-} 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.

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actively reabsorb NaCl across mTALs when Po2 drops below the critical pressure. How would our predictions differ in the absence of anaerobic metabolism (i.e., if the parameter $a$ 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. CNO profiles in the interstitium of the 4 concentric regions (R1–R4). In the base case (solid curves), $P_{NO}$ remains constant, and aquaporin-1 (AQP1) is permeable to NO. In the second case (dotted curves), $P_{NO}$ is taken to increase with decreasing Po2. In the third case (dash-dotted curves), AQP1 is taken to be impermeable to NO.

Fig. 6. CO2/H2O profiles in the interstitium of the 4 concentric regions (R1–R4), assuming a fixed $P_{NO}$. The solid and dashed curves, respectively, depict CO2 with and without O2 scavenging by NO, assuming that the O2 generation rate ($G_{O2}$) decreases with decreasing Po2. The dotted and dash-dotted curves, respectively, depict CO2 with and without O2 scavenging by NO, assuming that $G_{O2}$ is enhanced rather than limited by low medullary Po2. CO2 increases moderately in the absence of the NO–O2 reaction because SOD is the main O2 scavenger.
Specifically, assuming that \( a = 0 \), osm\( \text{CD} \) 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\( \text{CD} \) 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\( \text{CD} \) (464 mosmol/kgH\(_2\)O if \( a = 0 \), vs. 493 if \( a = 0.5 \)).

**AQP1-Mediated NO Transport**

The base case assumes that AQP1 transports NO, as observed experimentally (21). Nonetheless, there is some controversy as to whether AQP1 is indeed permeable to small gases such as CO\(_2\), NH\(_3\), and NO (57). We performed simulations in which AQP1 was taken to be impermeable to NO: the NO permeability of the vessels and tubules that express AQP1 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 AQP1, 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\(_{\text{NO}}\) 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\( \text{CD} \) 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<sub>CD</sub> by 18%, from 787 to 648 mosmol/kgH<sub>2</sub>O.

When inlet volume flows and maximal NO synthesis rates (i.e., \( G_{i,NO}^{max} \) in Eq. 15) are both increased by 20%, \( C_{NO} \) rises further, and osm<sub>CD</sub> is predicted to drop even more, to 575 mosmol/kgH<sub>2</sub>O (Table 6). When inlet volume flows, NO synthesis rates, and O<sub>2</sub> synthesis rates (i.e., \( G_{i,O2}^{basal} \) in Eq. 22a) are all increased by 20%, O<sub>2</sub> exerts greater compensating effects on mTAL transport, and osm<sub>CD</sub> climbs slightly, to 591 mosmol/kgH<sub>2</sub>O (Table 6). Together, these results suggest that an increase in tubular and vascular flows substantially reduces the OM axial osmolality gradient.

Fig. 8. \( C_{NO} \) 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<sub>2</sub> does not stimulate mTAL NaCl reabsorption (dash-dotted curves). Profiles were obtained assuming that \( P_{NO}^{rbc} \) is constant and that \( G_{O2} \) varies in parallel with P<sub>O2</sub>.

Fig. 9. O<sub>2</sub> 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<sub>2</sub> does not stimulate mTAL NaCl reabsorption (dash-dotted curves). Profiles were obtained assuming that \( P_{NO}^{rbc} \) is constant and that \( G_{O2} \) varies in parallel with P<sub>O2</sub>. 
F992 NITRIC OXIDE AND SUPEROXIDE MODULATE NaCl TRANSPORT AND Po2

Table 6. Effects of luminal flow and O2 synthesis on the concentrating capacity of the OM

<table>
<thead>
<tr>
<th>Condition</th>
<th>osmol(_{\text{CD}})</th>
<th>mosmol/kgH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Case</td>
<td>787</td>
<td></td>
</tr>
<tr>
<td>Without anaerobic metabolism</td>
<td>621</td>
<td></td>
</tr>
<tr>
<td>Without anaerobic metabolism and mTAL</td>
<td>655</td>
<td></td>
</tr>
<tr>
<td>stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without facilitated NO transport via AQP1</td>
<td>464</td>
<td></td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows</td>
<td>648</td>
<td></td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows and GNO</td>
<td>575</td>
<td></td>
</tr>
<tr>
<td>With a 20% increase in inlet water flows and Gno</td>
<td>591</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}})</td>
<td>1,073</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}}) and a 25% decrease in GNO</td>
<td>1,175</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}}) and a 50% decrease in GNO</td>
<td>1,297</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}}) and a 50% decrease in TNa/O2</td>
<td>935</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}}), a 50% decrease in TNa/O2, and a 25% decrease in GNO</td>
<td>1,020</td>
<td></td>
</tr>
<tr>
<td>With a 10-fold increase in G(_{\text{O2-}}), a 50% decrease in TNa/O2, and a 50% decrease in GNO</td>
<td>1,124</td>
<td></td>
</tr>
</tbody>
</table>

AQP1, aquaporin-1; G\(_{\text{NO}}\) and G\(_{\text{O2-}}\) are the volumetric rates of NO and O2 synthesis, respectively; TNa/O2, ratio of transported sodium to oxygen consumption. In all these simulations, G\(_{\text{O2-}}\) is taken to decrease with decreasing Po2, and P\(_{\text{NO}}\) 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\(_{\text{O2-}}\), with a subsequent 40% decrease in medulary blood flow and a nearly 20-mmHg increase in blood pressure (37). To dissect some of the underlying mechanisms, we raised maximal O2 generation rates in the OM by a factor of 10. As expected, the higher levels of O2 stimulate NaCl reabsorption and O2 consumption, thereby reducing Po2 and C\(_{\text{NO}}\). In the interbundle region, Po2 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 O2 concentration raises osmol\(_{\text{CD}}\) by ~300 mosmol/kgH2O, i.e., by 35% (Table 6). The increase is limited because 1) the effects of O2 on NaCl reabsorption across the mTAL 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 osmol\(_{\text{CD}}\) is predicted assuming that the number of Na\(^+\) moles actively reabsorbed per mole of O2 consumed (i.e., the mTAL TNa/O2 ratio) remains fixed at 18. There is some evidence, however, that NO and/or reactive oxygen species (ROS) modulate the amount of O2 consumed per Na\(^+\) ion transported, as discussed below. However, the explicit impact of NO and ROS on TNa/O2 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 O2 concentrations increase 10-fold, our model then predicts a smaller (20%) increase in osmol\(_{\text{CD}}\) (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 O2 levels. As expected, the greater the reduction in G\(_{\text{NO}}\), the faster NaCl transport across mTALs, and the higher the concentrating capacity of the OM (Table 6). The impact of the G\(_{\text{O2-}}\) increase, G\(_{\text{NO}}\) decrease, and TNa/O2 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 corticomedullary junction (x = 0). If the O2\(_{-}\) synthesis rate is multiplied by 10 and Na\(^+\) reabsorption thereby stimulated, it decreases much more rapidly. If TNa/O2 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\(_{\text{NO}}\), respectively. If TNa/O2 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 O2. 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 O2, NO, and O2\(_{-}\), which are summarized in Fig. 11.

The net production rate of NO and O2\(_{-}\) is oxygen dependent. Reciprocally, NO and O2\(_{-}\) affect oxygen availability in two ways: by modulating active transport across mTAL cells and therefore O2 consumption, and by regulating vessel contraction and therefore O2 supply. Our current model does not take into

Fig. 10. Na\(^+\) flow (in pmol·s\(^{-1}\)·tubule\(^{-1}\)) along the LAL. The thick arrow indicates the direction of the flow, from the OM-inner medullary (IM) boundary (x/L = 1) to the corticomedullary junction (x/L = 0). The black dotted line represents the base case. In all other cases, G\(_{\text{O2-}}\) is multiplied 10-fold to mimic hypertensive conditions. The ratio of transported sodium to oxygen consumption (TNa/O2) is taken as 18 (black curves) or 9 (grey curves), and the NO synthesis rate (G\(_{\text{NO}}\)) is either maintained constant (dashed lines) or halved (solid lines). NaCl reabsorption is maximal when the Na\(^+\) flow at x = 0 is the lowest.
consideration vessel diameter changes, i.e., vasoactive effects are not accounted for in this study. In the absence of such vasoactive effects, our model predicts that O$_2$-mediated stimulation of NaCl reabsorption raises the OM concentrating capacity (evaluated as the tubular fluid osmolality in collecting ducts at the OM-IM boundary) by >50% under basal conditions. Note that the numerical values in this study are necessarily approximations, given the significant uncertainty associated with several key parameters (see above). Conversely, NO-induced inhibition of NaCl reabsorption is predicted to decrease the OM concentrating capacity by >50% (Table 5). Our prediction that active transport of NaCl would substantially increase in the absence of NO is predicated on the hypothesis that an aerobic metabolism can supply a significant fraction of the energetic requirements of mTALs. Without glycolysis, NaCl transport would only increase by 5% in the absence of NO (Table 6), because there isn’t enough O$_2$ in the interbundle region to support per se significantly greater metabolic needs.

If NO didn’t inhibit NaCl active transport across mTALs, faster transport rates and therefore enhanced O$_2$ consumption would reduce PO$_2$, which in turn would lower NO production. The resulting decrease in CNO should then lead to vasoconstriction, which would further reduce PO$_2$ by limiting O$_2$ 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 PO$_2$. Similarly, activation of K$_{ATP}$ channels due to reduction of intracellular ATP might hyperpolarize vasa recta pericytes to favor vasodilatation (5).

Conversely, without O$_2$-mediated stimulation of sodium reabsorption and O$_2$ consumption, elevation of PO$_2$ would favor a rise in CNO and vasodilation, tending to enhance O$_2$ supply. In this case, however, active transport by the mTAL would also tend to increase, since it is partly limited by O$_2$ availability under basal conditions. Thus a rise in mTAL O$_2$ consumption would be favored, tending to offset the increase in O$_2$ supply. Finally, it seems plausible that other paracrine agents such as endothelins and vasoconstrictor prostaglandins might be released to limit such NO-dependent vasodilation. Given the complex nature of events that balance O$_2$ supply and consumption, we formulated the current model to facilitate prediction of the net effect of such interactions.

**Impact of NO on O$_2$**

Our model predicts that NO scavenging reduces O$_2$ levels by ~10% in the renal medulla. As recently observed by Hong and Garvin (25), flow-induced enhancement of O$_2$ 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 O$_2$ production appears to be mediated via the cGMP/PKG pathway (25). These novel findings suggest that NO and O$_2$ 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 O$_2$ production. In the absence of data, we did not incorporate this pathway in our model.

**Impact of O$_2$ on NO Bioavailability**

The impact of basal O$_2$ 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 O$_2$ levels. In a recent mathematical model of NO-O$_2$ interactions in OM cross sections (17), we showed that if O$_2$ is present in subnanomolar concentrations, it affects NO to a small extent only. In the current study, we assumed that O$_2$ is present at higher levels (1–10 nM), based on measured H$_2$O$_2$ concentrations. Thus C$_{NO}$ increased significantly when the NO-O$_2$ reaction rate was set to zero (Fig. 7). Moreover, when the effects of O$_2$ on mTAL reabsorption were abolished, C$_{NO}$ increased even more in the interbundle region (Fig. 8). In other words, the present model indicates that O$_2$ modulates NO levels both directly and indirectly: O$_2$ may indirectly reduce NO generation via its stimulation of mTAL metabolic requirements, leading to reduction of O$_2$ 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 G$_{NO}$ 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 O$_2$ 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 H$_2$O$_2$, 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 O$_2$ 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^O^-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 ~35%, and reduces Po$_2$ in the peripheral regions by 5–10 mmHg in the OS and 1–3 mmHg in the IS, assuming that the mTAL T$_{\text{Na}}$/Q$_{\text{O}_2}$ 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 (59). As discussed by Welch (58), possible explanations for the lower T$_{\text{Na}}$/Q$_{\text{O}_2}$ involve 1) oxidative stress-mediated changes in the Na$^+$ reabsorption profile, such that more Na$^+$ is reabsorbed downstream of the proximal tubule, at a higher energy cost; 2) back-leak of Na$^+$ in the tubular lumen; and 3) reduced efficiency of mitochondria in producing ATP in the presence of lower NO concentrations; note that NO modulates the respiration rate by inhibiting cytochrome oxidase in competition with O$_2$ (2). The extent to which oxidative stress reduces T$_{\text{Na}}$/Q$_{\text{O}_2}$ specifically in the mTAL remains to be ascertained. A twofold reduction would partly counteract the stimulating effects of O$_2^-$ on NaCl reabsorption across the mTAL (Fig. 10), but would further reduce Po$_2$ by a few millimeters mercury outside the vascular bundle. These results suggest that the inefficient use of O$_2^-$ in hypertensive models slows down active transport while exacerbating hypoxia in the OM.

**Tubular and Vascular Flow Increases**

We examined the net effect of increased flow on NaCl transport in the OM, given that luminal flow stimulates O$_2^-$ production, via PKC-mediated activation of NADPH oxidase (26), but also induces NOS activation in DVR and TALs. Shear stress enhances the intrinsic activity of NOS at least partly by stimulating its translocation to the membrane (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$_{\text{CD}}$ by 10%. The current model suggests that increasing volume flows may in fact reduce the osmolality 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 20% increase in the production of NO and O$_2^-$ is predicted to lower osm$_{\text{CD}}$ by ~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 Po$_2$ 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 Po$_2$) increased by ~40% following administration of L-NAME. Similarly, we found that abolishing NO generation reduced interstitial Po$_2$ 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 Po$_2$ 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 ~10 times higher, based on experimental determinations of medullary H$_2$O$_2$.

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$_2$O$_2$ (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$_2$O$_2$ 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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