Nitric oxide and superoxide transport in a cross section of the rat outer medulla. I. Effects of low medullary oxygen tension

Aurélie Edwards¹ and Anita T. Layton²

¹Department of Chemical and Biological Engineering, Tufts University, Medford, Massachusetts; and ²Department of Mathematics, Duke University, Durham, North Carolina

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Edwards A, Layton AT. Nitric oxide and superoxide transport in a cross section of the rat outer medulla. I. Effects of low medullary oxygen tension. Am J Physiol Renal Physiol 299: F616–F633, 2010. First published June 9, 2010; doi:10.1152/ajprenal.00680.2009.—To examine the impact of the complex radial organization of the rat outer medulla (OM) on the distribution of nitric oxide (NO), superoxide (O$_2^-$) and total peroxynitrite (ONOO), we developed a mathematical model that simulates the transport of those species in a cross section of the rat OM. To simulate the preferential interactions among tubules and vessels that arise from their relative radial positions in the OM, we adopted the region-based approach developed by Layton and Layton (Am J Physiol Renal Physiol 289: F1346–F1366, 2005). In that approach, the structural organization of the OM is represented by means of four concentric regions centered on a vascular bundle. The model predicts the concentrations of NO, O$_2^-$, and ONOO in the tubular and vascular lumen, epithelial and endothelial cells, red blood cells (RBCs), and interstitial fluid. Model results suggest that the large gradients in O$_2$ from the core of the vascular bundle toward its periphery, which stem from the segregation of O$_2$-supplying descending vasa recta (DVR) within the vascular bundles, in turn generate steep radial NO and O$_2^-$ concentration gradients, since the synthesis of both solutes is O$_2$ dependent. Without the rate-limiting effects of O$_2$, NO concentration would be lowest in the vascular bundle core, that is, the region with the highest density of RBCs, which act as a sink for NO. Our results also suggest that, under basal conditions, the difference in NO concentrations between DVR that reach into the inner medulla and those that turn within the OM should lead to differences in vasodilation and preferentially increase blood flow to the inner medulla.

Mathematical model; rat kidney; generation rates; peroxynitrite; thick ascending limb sodium transport

BLOCKING THE SYNTHESIS of nitric oxide (NO) in the renal medulla leads to a reduction in blood flow, salt retention, and hypertension (12, 36, 40). NO and its scavenger superoxide (O$_2^-$) both modulate renal medullary vascular and tubular function, albeit in opposite ways. Whereas NO inhibits tubular sodium reabsorption and increases medullary blood flow by promoting the vasodilation of descending vasa recta (DVR), O$_2^-$ enhances medullary thick ascending limb (mTAL) Na$^+$ reabsorption (19) and acts to reduce medullary blood flow by mechanisms that have yet to be fully elucidated (17). The product of the NO-O$_2^-$ reaction, ONOO$^-$, is thought to inhibit Na$^+$ reabsorption (19). In vitro experiments suggest that the interactions between O$_2^-$ and NO ultimately determine the effectiveness of tubulovascular cross talk (39). The overall objective of this study was to investigate the impact of the radial organization of the outer medulla (OM) of the rat kidney on the interactions between NO and O$_2^-$. In the current study, we examined the effects of low medullary PO$_2$ on the distribution of NO, O$_2^-$, and total peroxynitrite (the sum of ONOO$^-$ and ONOOH, denoted ONOO). In a companion study (15), we considered the effects of kinetic and transport rates on the concentration profiles of these species, and the importance of tubulovascular cross talk and of NO-O$_2^-$ interactions in vivo.

Anatomic studies have revealed that the medullary organization of tubules and vessels is highly structured in a number of mammals (29), including rats and mice (28, 30). DVR and ascending vasa recta (AVR) form tightly packed vascular bundles that appear to dominate the histotopography of the OM, especially in the inner stripe. Throughout the OM, collecting ducts (CDs) are found distant from vascular bundles, whereas the loops of Henle are positioned nearer the bundles. Modeling studies have shown that the structural organization results in preferential interactions among tubules and vasa recta (33, 49). In an earlier modeling study, we examined the impact of the structural organization of the rat OM on O$_2$ distribution (5). We found that the segregation of DVR, the main supply of O$_2$, at the center and immediate periphery of the vascular bundles gives rise to large radial differences in Po$_2$. In the current study, we developed a mathematical model that simulates the generation, transport, and consumption of NO, O$_2^-$, and ONOO in cross sections of the rat OM. We had previously built a model of NO transport in a cross section of the OM. (A total of 154 tubules and 230 vasa recta were individually represented in the ZE model.) In contrast, the present model, which accounts for the preferential interactions among tubules and vessels using the region-based approach developed by Layton and Layton (33), represents only one model tubule or vessel for each class of tubules or vessels. The radial position of the tubules and vessels is specified by assigning them to one of the four concentric regions; the portion of each concentric region that is exterior to both tubules and vasa recta represents interstitial cells, merged capillary plasma, and interstitial space. Since the representation of the position of individual structures is less detailed using the region-based approach, the present model requires substantially less computational time than the ZE model. This has allowed us to introduce more complexity in other ways. First, in the present model, the concentrations of O$_2^-$ and ONOO are explicitly calculated, whereas O$_2^-$ levels were arbitrarily fixed in the ZE model. Second, the present model
accounts for the effects of medullary hypoxia, which were neglected in the ZE model. Third, in contrast to the present model, the ZE model did not incorporate the OM capillary plexus. As described below, the addition of each of these new features has a significant impact on the predicted NO, O$_2^-$, and ONOO$^-$ concentration profiles.

**MODEL DESCRIPTION**

In this section, we describe a base-case model configuration, model equations, and a set of base-case model parameters, including physical dimensions and transport parameters. In addition, we briefly describe the numerical methodology used to obtain model solutions. Acronyms and symbols are given in the Glossary.

**Configuration and Radial Organization**

We seek to investigate the effects of the radial organization of the rat OM, and the resulting preferential interactions among tubules and vessels, on the distribution of NO and O$_2^-$. To achieve that goal, we use the region-based approach developed by Layton and Layton (33). In the region-based formulation, the structural organization of the OM is represented by means of four concentric regions centered on a vascular bundle: an innermost region containing the central vascular bundle (R1), where all the long DVR (i.e., DVR that reach into the inner medulla) and one-third of the long AVR (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 thick ascending limbs, both long and short, and some short AVR; and the region most distant from the vascular bundle (R4), where collecting ducts (CDs) and the remaining short AVR are located. Descending limbs that reach into the inner medulla are situated 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.

We consider the cross sections at two axial positions of the OM: one at the mid-OS (0.3 mm from the corticomedullary boundary in a typical rat OM that is 2.1 mm long) and another at the mid-IS (1.35 mm from the corticomedullary boundary). These two positions are chosen because the radial positions of some of the tubules and vessels differ substantially in the OS and in the IS (see above). Also, in a model of O$_2$ transport in the OM (5), the PO$_2$ in the IS was predicted to be substantially lower than in the OS, so a comparison of the model predictions for the two cross sections should illustrate the effects of O$_2$ availability on the distribution of NO and O$_2^-$. The radial organization of tubules and vasa recta with respect to vascular bundles is represented by specifying the fractions of the tubules and vasa recta assigned to each concentric region at each medullary level. Figure 1 shows the relative positions of tubules and vessels in a cross section through the OS (Fig. 1A) and through the IS (Fig. 1B).

To simulate the distribution of NO, O$_2^-$, and ONOO$^-$ in a cross section of the OM, we distinguish between the concentrations of NO, O$_2^-$, and ONOO$^-$ at the mid-IS (1.35 mm from the corticomedullary boundary) and another position in the OS (0.3 mm from the corticomedullary boundary). NO and O$_2^-$ are generated within vascular endothelia and tubular epithelia but not within the lumen, and the rates of NO consumption in red blood cells (RBCs) and plasma differ by orders of magnitude (see below). Hence, to accurately represent the generation and consumption of NO and O$_2^-$, we distinguish between the lumen and the surrounding epithelium in each tubule, and between the RBC compartment, the plasma compartment, and the surrounding endothelium in each vas rectum, as illustrated in Fig. 1C. The interstitia of each of the four concentric regions also constitute separate compartments. The capillaries that traverse radially across the OM cross sections are also represented. Given that the properties (e.g., dimensions and permeabilities) of the medullary capillary network have not been well documented, capillary plasma is assumed to be well mixed with the local interstitium. In this model, the capillaries thus consist of two compartments, which exchange directly with each other: the capillary RBCs and the surrounding capillary endothelium. The solute concentrations and reaction rates associated with the capillary RBC cytosol and the capillary endothelium in a given region (R1–R4) represent radial averages in that region.

**Solute Represented in the Model**

The model is formulated for three solutes, NO, O$_2^-$, and ONOO$^-$; the latter represents the total amount of peroxynitrite (ONOO$^- +$ ONOOH). Hemoglobin concentrations in RBCs, and PO$_2$ in the lumen, RBC, and interstitial fluid are set a priori, based on the predictions of an O$_2$ model (4). That O$_2$ model does not represent the endothelium and epithelium explicitly; thus, in the current study, the PO$_2$ values in the endothelial and epithelial compartments are taken as the average between interstitial and luminal PO$_2$. The present model predicts the concentrations of NO, O$_2^-$, and ONOO$^-$ (as well as the associated fluxes) in the tubular and vascular lumen, epithelial and endothelial cells, RBCs, and interstitial fluid (i.e., a total of 40 compartments).

**Conservation Equations**

Solute concentrations are determined by solving conservation equations in each compartment. At steady state, the conservation of solute $k$ ($k = \text{NO}, \text{O}_2^-, \text{or ONOO})$ in a given vascular endothelium or tubular epithelium (denoted “cell”) is given by

$$0 = \psi_k^\text{cell} A_{\text{cell}} + j_{k}^{\text{lumen, cell}} - \sum_{R=R1,R4} j_{k}^{\text{cell, R}}$$

where $\psi_k^\text{cell}$ is the volumetric rate of net production (i.e., generation minus consumption) of solute $k$ in compartment $i$, $A_i$ is the cross-sectional area of compartment $i$, $j_{k}^{\text{lumen, cell}}$ is the flux of solute $k$ from the vascular or tubular lumen into the surrounding cellular layer, and $j_{k}^{\text{cell, R}}$ is the flux of solute $k$ from the cellular layer into the surrounding interstitium of region R. The latter may be zero if the tubule or vessel does not reside in R. For example, for the short descending limb, $j_{k}^{\text{cell, R4}}$ is non-zero in the OS, where the limb straddles R3 and R4, but is null in the IS, where the limb straddles R2 and R3, but not R4 (see Fig. 1).

Conservation of solute $k$ within the tubular lumen is expressed as

$$0 = \psi_k^{\text{lumen}} A_k^{\text{lumen}} - j_{k}^{\text{lumen, cell}}$$

In the above equation, axial advective flux is neglected because its magnitude is believed to be sufficiently small compared...
with the synthesis rate or the transmembrane flux (52). In vasa recta lumen, we distinguish between the RBC compartment (denoted “RBC”) and the outer, plasma layer (denoted “pl”). Conservation of solute \( k \) within these two compartments is written as

\[
0 = \Psi_k^{RBC} A_{RBC} - J_k^{RBC,pl} \tag{3a}
\]

\[
0 = \Psi_k^{pl} A_{pl} + J_k^{RBC,pl} - J_k^{pl,cell} \tag{3b}
\]

Conservation of solute \( k \) within the capillary RBC cytosol (denoted “cap RBC”) and the surrounding endothelium (denoted “cap endo”) is expressed as

\[
0 = \Psi_k^{capRBC} A_{capRBC} - J_k^{capRBC, cap endo} \tag{4a}
\]

\[
0 = \Psi_k^{cap endo} A_{cap endo} + J_k^{cap RBC, cap endo} - J_k^{cap endo, R} \tag{4b}
\]

Axial advective flux is also neglected in these equations, as in Eq. 2. Following the approach of Layton and Layton (33), solute conservation in the interstitium of each concentric region is expressed as

\[
0 = \Psi_k^{R AVR} A_{int R} + J_k^{totR} + C_k^{SDV} Q_{SDV, R} - C_k^R Q_{AVR, R} - C_k^R Q_{R, R} \tag{5}
\]

The first term represents the net production of solute \( k \) within the interstitium of region \( R \); \( A_{int R} \) is the area occupied by interstitium in region \( R \) (\( R = R1, R2, R3, \) and \( R4 \)). The second term, \( J_k^{totR} \), is the total flux of solute \( k \) into region \( R \) from tubules, vasa recta, capillaries, and adjoining regions. The third term represents the capillary source term, where SDV denotes short DVR, \( C_k^{SDV} \) is the concentration of solute \( k \) in SDV, and \( Q_{SDV, R} \) is the rate of blood flow from SDV into region \( R \). Although water fluxes are not explicitly computed in this model, they are incorporated as parameters, using values predicted by a previous model (4), so that the roles of AVR and capillary sinks in the conservation of solutes can be represented. In a given region, the sum of the capillary source flows and water fluxes emanating from tubules and vessels equals the total fluid reabsorbed into that region; at steady state, there is no fluid accumulation, that is, the fluid reabsorbed is drained away in its entirety, via entry into AVR and capillary transport.
into surrounding regions. The fourth term in Eq. 5 represents the solute that is carried away by the AVR located in region R; \( Q_{AVR,R} \) is the fluid flow entering the AVR in R. The last term represents the solute that is carried by capillary flow into an adjoining region \( R' \); \( Q_{R,R'} \) is the fluid flow from region R into region \( R' \).

**Flux Calculations**

Across most barriers, water and solutes do not share the same pathway, so that the solute flux is driven only by diffusion [exceptions include NO fluxes across aquaporin-1 (AQP1) water channels in DVR endothelium and descending limb epithelium, as noted below]. In this case, the transmural flux (in mol·s\(^{-1}·\text{m}^{-2} \)) of solute \( k (k = \text{NO, } \text{O}_2^-, \text{ or } \text{ONOO}) \) from compartment \( i \) into compartment \( j \), taken positive into compartment \( j \), can be written as

\[
J_{k}^{i,j} = 2 \pi R_{i,j} \left[ C_{i}^{k} - C_{j}^{k} \right]
\]

For a tubule, \( i \) is lumen and \( j \) is epithelium; for a vessel, \( i \) is plasma and \( j \) is endothelium, or \( i = \text{RBC and } j = \text{plasma} \); for capillaries, \( i = \text{RBC cytosol} \) and \( j = \text{endothelium} \). In Eq 6, \( R_{i,j} \) is the radius at the \( i-j \) interface, \( P_{k}^{i,j} \) is the permeability of the \( i-j \) interface to solute \( k \), and \( C_{i}^{k} \) and \( C_{j}^{k} \) are the concentrations of solute \( k \) in compartments \( i \) and \( j \). Some vessels and tubules straddle several regions (Fig. 1), so the transmural flux of solute \( k \) from the vascular endothelium or tubular epithelium into a surrounding region \( R \) is calculated as (33)

\[
J_{k}^{\text{cell,}R} = 2 \pi R_{\text{cell,R}} R_{\text{cell,R}} C_{\text{cell,R}}^{k} \left( C_{\text{cell,R}}^{k} - C_{R}^{k} \right)
\]

where \( R_{\text{cell,R}} \) is the outer radius of the cellular layer, and \( \kappa_{\text{cell,R}} \) is the fraction of the vascular endothelium or tubular epithelium in contact with region \( R \), such that \( \sum_{R' = \text{R1}-\text{R4} \cap \text{cell,R}} R_{\text{cell,R}} = 1 \). For example, for the long ascending limb in the OS, \( \kappa_{\text{cell,R1}} = 0 \), \( \kappa_{\text{cell,R2}} = 0.5 \), \( \kappa_{\text{cell,R3}} = 0.5 \), and \( \kappa_{\text{cell,R4}} = 1 \).

Across AVR fenestrations, water is reabsorbed directly from the interstitium into the AVR lumen, and solutes are carried along. The convective-diffusive flux of solute \( k (k = \text{NO, } \text{O}_2^-, \text{ or } \text{ONOO}) \) from the AVR lumen (“lum”) into the interstitium of the surrounding region \( R \) (“\( R' \))”, is expressed as

\[
J_{k}^{\text{fen}} = J_{k}^{\text{fen}} \left[ C_{\text{lum}}^{k} - C_{R}^{k} \exp(-P_{k}^{\text{fen}}) \right] \left[ 1 - \exp(-P_{k}^{\text{fen}}) \right]
\]

and between DVR compartments \( i \) and \( j \) \( (i = \text{plasma and } j = \text{endothelium}, \text{or } i = \text{RBC and } j = \text{plasma}) \), is expressed as

\[
J_{k}^{i,j} = J_{k}^{j,i} \left[ \frac{C_{i}^{k} - C_{j}^{k} \exp(-P_{k}^{j,i} \exp(-P_{k}^{i,j}))}{1 - \exp(-P_{k}^{i,j})} \right]
\]

where \( J_{k}^{i,j} \) denotes the volume flux (in \( \text{m}^2/\text{s} \)) between \( i \) and \( j \), and \( P_{k}^{i,j} \) is the Pécelet number associated with compartment \( i \) and \( j. \) \( J_{NO}^{i,j} \) is the flux of NO between descending limb compartments \( i \) and \( j \). Similarly, the flux of NO from the descending limb epithelium or the DVR endothelium (denoted \( i \)) into region \( R \) is calculated as

\[
J_{k}^{i,R} = J_{k}^{R,i} \left[ \frac{C_{i}^{k} - C_{R}^{k} \exp(-P_{k}^{R,i} \exp(-P_{k}^{R,i}))}{1 - \exp(-P_{k}^{R,i})} \right]
\]

The total flux of solute \( k \) \( (k = \text{NO, } \text{O}_2^-, \text{ or } \text{ONOO}) \) into region \( R \) is then given by (33)

\[
J_{k}^{R} = \sum_{i} n_{i} J_{k}^{i,R} + 2 \pi \sum_{R' \cap R} R_{R',R} P_{k}^{R,R'} (C_{R'}^{k} - C_{R}^{k})
\]

The first term on the right-hand side represents the sum of solute fluxes from tubular epithelia, vascular endothelia, and capillary endothelia (indexed by \( i \)) into \( R \). The term \( J_{k}^{R,R'} \) represents the diffusive solute flux into \( R \) from adjacent regions \( R' \). The term \( J_{k}^{R,R'} \) represents the effective permeability to solute \( k \) of the boundary separating regions \( R \) and \( R' \). Effective permeability takes into account the diffusion resistance that represents the effects of macromolecules and interstitial cells in the interstitium, and the effect of tortuosity on the diffusion path length around tubules and vessels (see below).

**Vascular, Tubular, and Interregion Permeabilities**

Estimates of the RBC membrane permeability to NO (\( P_{NO}^{\text{RBC}} \)) vary over a wide range, from 400 to 450 \( \mu \text{m/s} \) (16, 47), to 45,000 or 64,000 \( \mu \text{m/s} \) (25, 35). In this study, the plasma and RBC compartments are taken to be well mixed. To account for the diffusive resistance within these layers, the base-case value of \( P_{NO}^{\text{RBC}} \) is set to 1,000 \( \mu \text{m/s} \) (0.1 \text{cm/s}), that is, in the lower range.

Herrera and colleagues (21, 22) recently found that AQP1 water channels transport NO and thereby regulate endothelium-dependent vasorelaxation. Given that DVR, RBCs, proximal tubules, and thin descending limbs all express AQP1, NO mediated transport may significantly affect NO levels throughout the medulla. In Chinese ovary K1 cells stably transfected with an AQP1 expression vector, Herrera et al. (22) observed a linear relationship between the water permeability (\( P_{w} \), in \( \mu \text{m/s} \)) and the NO permeability (\( P_{NO}^{\text{AQP1}}, \) in fluorescence units per time)
We use this relationship to estimate the NO permeability of the endothelial or epithelial layer surrounding the lumen in vessel or tubule \( P_{i, NO} \), given the RBC permeability to NO

\[
P_{i, NO}^\text{cell} = P_{RB/C NO} \left( \frac{0.64 P_{RB/C NO} + 20.23}{0.64 P_{RB/C NO} + 20.23} \right)
\]

where \( P_{i, NO}^\text{cell} \) is the water permeability of the endothelium or epithelium. In the vessels and tubules \( i \) that do not express AQP1 (i.e., AVR, ascending limbs, and CDs) \( P_{i, NO}^\text{cell} \) is taken as zero in Eq. 13.

In the current model, the cellular layers are modeled as compartments, not as single barriers. To estimate the NO permeability at the interface between the cellular layer of vessel or tubule \( i \) and the surrounding interstitium (denoted \( P_{i, NO}^\text{cell-int} \)), and the NO permeability at the interface between the lumens of tube \( i \) (or the plasma of vessel \( i \)) and the surrounding cell layer (denoted \( P_{i, NO}^\text{lumen-cell} \)), we apply the concept of transmembrane NO permeability \( P_{i, NO}^\text{cell-int} \) (from Eq. 13), we can estimate \( P_{i, NO}^\text{cell-int} \) and \( P_{i, NO}^\text{lumen-cell} \). In a given vessel \( i \), the NO permeability at the plasma-RBC interface (denoted \( P_{RB/C NO}^\text{cell} \)) is given by \( P_{RB/C NO} \).

In the absence of data for the tubular and vascular permeability to \( O_2^- \), the latter is estimated based upon the \( O_2^- \) diffusivity across membranes (\( D_{O_2^-}^{\text{memn}} \)). The \( O_2^- \) permeability at the interface between the cellular layer of vessel or tubule \( i \) and the surrounding interstitium (denoted \( P_{i, O_2^-}^{\text{cell-int}} \)) and that at the interface between the lumen of tube \( i \) (or the plasma of vessel \( i \)) and the surrounding cell layer (denoted \( P_{i, O_2^-}^{\text{lumen-cell}} \)) are calculated as

\[
P_{i, O_2^-}^{\text{cell-int}} = P_{i, O_2^-}^{\text{lumen-cell}} = \frac{D_{O_2^-}^{\text{memn}}}{(L_{\text{cell}})/2}
\]

where \( L_{\text{cell}} \) is the thickness of the epithelial or endothelial layer. Similarly, the \( O_2^- \) permeability at the RBC-plasma interface is determined as \( P_{RB/C NO}^{\text{RB/pl}} = D_{O_2^-}^{\text{memn}}/(L_{\text{RB}}/2) \), where \( L_{\text{RB}} \) is the thickness of the RBC compartment. We use an identical approach to determine the permeabilities to ONOO. The diffusivity of \( O_2^- \) and ONOO at \( 37^\circ C \) in dilute solution has been reported as 2,800 and 2,600 \( \mu m^2/s \), respectively.

The ability of erythrocytes to release NO under hypoxic conditions has given rise to two hypotheses, according to which SNOHb or RBC nitrite constitutes a pool of bioavailable NO (13, 42). Following the approach of Chen et al. (6, 8), the volumetric generation rate of NO via the SNOHb and nitrite pathways is calculated as, respectively

\[
\Gamma_{\text{SNOHb}} = k_{\text{SNOHb}}^\text{RBC} \cdot \text{SNOHb}
\]

\[
\Gamma_{\text{nitrite}} = k_{\text{nitrite}}^\text{RBC} \cdot \text{nitrite} \cdot \text{Hb}
\]

where Hb denotes deoxyhemoglobin. The form under which the NO thereby formed is exported out of the RBC cytosol remains uncertain. If it were released into the RBC cytosol, it would immediately react with hemoglobin and would not have
any vasodilatory effect. Thus Chen and colleagues (6, 8) postulated that the RBC membrane possesses a mechanism that facilitates the export of NO out of the cell, or that another NO-related species is the immediate product of these two reactions, and they modeled the SNOHb- and nitrite-mediated NO release rate as a surface reaction at the RBC-plasma interface. Given our compartmental approach, we assume instead that the amount of NO thereby generated is released uniformly throughout the adjacent plasma layer. Hence, the volumetric NO generation rate in the plasma compartment is given by

\[ G_{\text{NO}}^\text{pl} = (\Gamma_{\text{NO}}^\text{SNOHb} + \Gamma_{\text{NO}}^\text{nitric})(A_{\text{RBC}}/A_{\text{pl}}) \]  

(19)

**NO Consumption Rates**

As in our previous model of NO transport (54), the NO consumption reactions considered here are the autoxidation of NO (rate \( v_1 \)), the scavenging of NO by \( O_2^- \) (rate \( v_2 \)), the irreversible reaction of NO with oxyhemoglobin (HbO\(_2\)) to form methemoglobin (rate \( v_3 \)), and the reversible reaction of NO with deoxyhemoglobin (Hb) to form HbNO (rate \( v_4 \))

\[
\begin{align*}
v_1 &= k_{\text{O}_2}(C_{\text{NO}})^2C_{\text{O}_2} \\
v_2 &= k_{\text{NO}^-\text{Sup}}C_{\text{NO}}C_{\text{O}_2^-} \\
v_3 &= k_{\text{oxy}}C_{\text{NO}}C_{\text{HbO}_2} \\
v_4 &= k_{\text{deoxy}}C_{\text{NO}}C_{\text{Hb}} - k_{\text{rev}}C_{\text{HbNO}} 
\end{align*}
\]

(20) in all compartments

(21) in RBCs only

(22) in RBCs only

(23)

**O\(_2\)\(^-\) Generation Rates**

Li et al. (34) measured the production of \( O_2^- \) in glomeruli and renal tubular segments; under well-oxygenated conditions (95% \( O_2 \)), the rates for the mTAL, the medullary CD, the thin limb of Henle’s loop, and the glomeruli were \( \approx 800, 400, 500 \), and 500 fluorescence units/mm\(^2\) respectively. In the absence of data for vasa recta, we assume that the \( O_2^- \) production rate in medullary vessels is similar to that in glomeruli. Given the lumen diameter and the thickness of the surrounding cellular layer, the rates per square millimeter are converted to volumetric rates, respectively yielding 0.071, 0.041, 0.342, and 0.473 fluorescence units/mm\(^3\) for the mTAL, the CD, the thin limb, and vasa recta. The vasa recta-to-mTAL ratio of \( O_2^- \) synthesis is therefore taken as 0.473/0.071, the vasa recta-to-CD ratio as 0.473/0.041, and the vasa recta-to-thin limb ratio as 0.473/0.342. When \( P_{O_2} \) is plentiful, the rate of \( O_2^- \) production in vasa recta endothelium is assumed to be equal to the measured rate of \( O_2^- \) production in aortic endothelial cells, that is, 0.7 \( \mu \text{M/s} \) \((43)\). Thus, under well-oxygenated conditions, the volumetric rate of \( O_2^- \) generation (denoted \( G_{O_2^-}^\text{high PO}_2 \)) is taken as 0.106, 0.061, and 0.508 \( \mu \text{M/s} \) in the epithelium of mTALs, OMCDs, and thin limbs, respectively.

The effects of low medullary \( P_{O_2} \) on the \( O_2^- \) generation rate remain to be fully understood. Chen et al. (11) observed that NADPH-dependent \( O_2^- \) production in kidney homogenates was reduced by 60–90% as \( P_{O_2} \) was decreased from 76 to 2 mmHg. The \( P_{O_2} \) value at which NADPH-dependent \( O_2^- \) production was halved in normotensive rats was estimated as 15.4 mmHg. On the other hand, Li et al. (34) found that exposure of cortical thick ascending limbs to low \( P_{O_2} \) (5–10 mmHg) significantly raised \( O_2^- \) production, by 35 and 65%, respectively (relative to well-oxygenated conditions), in the absence and presence of NADH. Whereas NAD(P)H oxidase is the major source of \( O_2^- \) under physiological conditions, other \( O_2^- \) synthesis pathways include xanthine oxidase, mitochondrial respiratory chain enzymes, and NOS. Hypoxia-induced stimulation of \( O_2^- \) production has been reported in other tissues. In the left ventricular myocardium, intermittent hypoxia was found to increase NADPH-dependent \( O_2^- \) production (38), while hypoxia increased xanthine oxidase activity in pulmonary artery endothelial cells (26, 46). Thus different sources of \( O_2^- \) may contribute to the increase in \( O_2^- \) under low-\( P_{O_2} \) conditions in certain cell types. Given these conflicting findings and the absence of cell-specific data in the renal medulla, we consider three different scenarios.

**Case A.** Under low-\( P_{O_2} \) conditions, the rate of \( O_2^- \) synthesis (denoted \( G_{O_2^-}^\text{low \( P_{O_2} \)} \) in the endothelium or epithelium of vessel or tubule \( i \)) is assumed to be rate limited by oxygen availability. The \( O_2^- \) dependence of the \( O_2^- \) generation rate is modeled using a Michaelis-Menten relationship (1)

\[
G_{O_2^-}^i = \frac{G_{O_2^-}^i \text{ high PO}_2}{P_{O_2} + K_{O_2^-}^i} \quad (25a)
\]

where \( K_{O_2^-}^i \) is taken as 15.4 mmHg, based upon the data of Chen et al. (11).

**Case B.** We assume that the rate of \( O_2^- \) synthesis remains independent of \( P_{O_2} \)

\[
G_{O_2^-}^i \text{ low \( P_{O_2} \))} = G_{O_2^-}^i \text{ high PO}_2 \quad (25b)
\]

**Case C.** \( O_2^- \) production under low-\( P_{O_2} \) conditions is taken to be 50% higher than under well-oxygenated conditions

\[
G_{O_2^-}^i \text{ low \( P_{O_2} \))} = 1.5 \cdot G_{O_2^-}^i \text{ high \( P_{O_2} \))} \quad (25c)
\]

That is, under physiological (i.e., hypoxic) conditions in the medulla, the volumetric rate of \( O_2^- \) production is taken as
1.05, 0.159, 0.092, and 0.762 μM/s in vasa recta, mTALs, OMCDs, and thin limbs, respectively.

**O$_2^-$ Consumption Rates**

The O$_2^-$ consumption reactions considered here are the scavenging reactions with NO (rate v5) and with superoxide dismutase, or SOD (rate v5). Both occur in every compartment, and the volumetric rate of the latter reaction is calculated as

$$v_5 = k_{SOD} \cdot C_{O_2^-} \cdot C_{SOD}$$

(26)

Thus the net production rate of O$_2^-$ in compartment $i$ is given by

$$\Psi_{O_2^-}^i = G_{O_2^-}^i - v_2^i - v_5^i$$

(27)

**ONOO Generation and Consumption Rates**

ONOO is formed by the reaction between NO and O$_2^-$ in RBC cytosol compartment is given by the product of the RBC cytosol compartment for a given region is computed as the product of the circumference of the capillary RBC cytosol compartment associated with a given region are taken to be the averages of the corresponding concentrations in the capillary RBC fluid of that region. Concentration values are obtained for the mid-OS and for the mid-IS. At the mid-OS, the flow of water into AV (i.e., the parameter $Q_{AVR, R}$ in Eq. 5) is calculated as $4.5 \times 10^{-7} \text{cm}^2 \text{s}^{-1} \text{bundle}^{-1}$ in R1, $2.8 \times 10^{-7}$ in R2, $3.4 \times 10^{-5}$ in R3, and $3.0 \times 10^{-5}$ in R4. The interregion flow of water (i.e., the parameter $Q_{R, R'}$ in Eq. 5) is calculated as $2.4 \times 10^{-8} \text{cm}^2 \text{s}^{-1} \text{bundle}^{-1}$ in R1, $5.4 \times 10^{-6}$ (R2 to R3), and $8.4 \times 10^{-6}$ (R3 to R4). At the mid-IS, $Q_{AVR, R}$ is determined as $1.1 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \text{bundle}^{-1}$ in R1, $1.5 \times 10^{-6}$ in R2, $2.3 \times 10^{-5}$ in R3, and $6.5 \times 10^{-6}$ in R4, whereas $Q_{R, R'}$ is determined as $5.6 \times 10^{-8} \text{cm}^2 \text{s}^{-1} \text{bundle}^{-1}$ (R1 to R2), $2.9 \times 10^{-5}$ (R2 to R3), and $5.7 \times 10^{-5}$ (R3 to R4).

The total concentration of heme species (Hb + HbO$_2$) also comes from our recent O$_2$ model (4); at the mid-OS and mid-IS, it ranges between 20 and 27 mM, depending on the vessel, except in the short AVR at the mid-IS, where it is ~34 mM. The concentration of HbNO is taken as 1 μM, an estimate in the midrange of reported values (24, 31); results were found to be insensitive to that parameter (not shown). The structural organization of the medulla is thought to result in radial pH and CO$_2$ concentration gradients (32). The pH is taken as 7.4 in R1 and R2, and as 7.2 in R3 and R4; the PCO$_2$ is taken as 40 mmHg (i.e., 1.2 mM) in R1 and R2 and 50 mmHg (i.e., 1.5 mM) in R3 and R4.

To compute solute fluxes across capillaries, the circumference of the capillary compartments is needed. The circumference of the capillary RBC cytosol compartment for a given region is computed as the product of the circumference of the capillary RBC tube (predicted by the model in Ref. 4) and the capillary path length within that region, then multiplied by the rate at which the short DVR turns at the appropriate medullary level (mid-OS or mid-IS). To compute net generation rates, the surface area of the capillary compartments is needed. The surface area of the capillary RBC cytosol compartment is also used to compute solute fluxes across capillaries. The capillary tube cross-sectional surface area and the capillary path length within that region, then multiplied by the rate at which the short DVR turns at that medullary level (mid-OS or mid-IS).

The thickness of the capillary endothelium is taken to be equal to that of the vasa recta endothelium (1 μm).

Parameters related to the physical properties and dimensions of vessels, tubules, and interstitial regions are derived from the region-based model of Layton and Layton (33) and are given in Tables 1 (vessels and tubules) and 2 (interstitial regions and capillaries). Reaction and diffusion rates are summarized in Table 3, volumetric generation rates and cross-sectional areas are given in Table 4, and permeabilities are shown in Table 5.

**Numerical Method**

The present model yields NO, O$_2^-$, and ONOO fluxes and concentrations in each of the 40 compartments considered herein. These fluxes and concentrations are determined by simultaneously solving conservation equations in each com-
Our previous study (5), the segregation of O2-rich long DVR severely limited by the availability of oxygen. As described in
Region parameters
Table 2. Concentration Profiles Under Well-Oxygenated Conditions

<table>
<thead>
<tr>
<th>Vessel or Tubule</th>
<th>*Inner Diameter, μm</th>
<th>Thickness, μm</th>
<th>No./Vascular Bundle</th>
<th>*Permeability to Water, μm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long descending vasa recta</td>
<td>11.0</td>
<td>1.0</td>
<td>12</td>
<td>1,257</td>
</tr>
<tr>
<td>Short descending vasa recta</td>
<td>11.0</td>
<td>1.0</td>
<td>Mid-OS: 43.4</td>
<td>1,257</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mid-IS: 21.4</td>
<td></td>
</tr>
<tr>
<td>Long ascending vasa recta</td>
<td>27.0/15.3</td>
<td>1.0</td>
<td>50(17 in R1, 33 in R2)</td>
<td>†</td>
</tr>
<tr>
<td>Short ascending vasa recta</td>
<td>27.0/15.3</td>
<td>1.0</td>
<td>Mid-OS: 288(216 in R3, 72 in R4)</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mid-IS: 142(106.5 in R3, 35.5 in R4)</td>
<td></td>
</tr>
<tr>
<td>Long descending limbs</td>
<td>80.0/8.6</td>
<td>1.1</td>
<td>23.7</td>
<td>3,570/2,295</td>
</tr>
<tr>
<td>Short descending limbs</td>
<td>20.0/18.3</td>
<td>1.1</td>
<td>47.3</td>
<td>3,570/3,257</td>
</tr>
<tr>
<td>Long ascending limbs</td>
<td>20.0</td>
<td>8.0</td>
<td>23.7</td>
<td>0</td>
</tr>
<tr>
<td>Short ascending limbs</td>
<td>20.4/13.2</td>
<td>8.0</td>
<td>47.3</td>
<td>0</td>
</tr>
<tr>
<td>Collecting ducts</td>
<td>30.5/24.7</td>
<td>9.0</td>
<td>11.5</td>
<td>463</td>
</tr>
</tbody>
</table>

Diameters, numbers, and permeabilities are taken from Ref. 33, and thicknesses from Ref. 5. The large diameter of the long descending limbs in the outer stripe (OS) represents the tortuosity of the proximal straight tubules. *When 2 values are given, the first one corresponds to the mid-OS, the second to the mid-inner stripe (mid-IS). Otherwise, diameters are taken to remain constant along the corticomedullary axis †Ascending vasa recta (AVR) water permeability is not given because AVR water fluxes are computed based on fluid accumulation into a region (R1–R4).

RESULTS

Using the model configuration and parameter set, the model equations were solved to obtain NO, O2, and ONOO concentrations in each compartment, as well as the solute fluxes between adjacent compartments (note that transmembrane fluxes can be related to, or inferred from, the difference in concentrations between two adjacent compartments). Simulations were performed for the mid-OS and mid-IS, and trends were generally found to be the same at these two axial positions. Hence, when mid-OS and mid-IS results do not differ substantially, only the latter are described. To assess the effect of low PO2 on the distribution of NO, O2, and ONOO, we first simulated a case in which the OM is assumed to be well oxygenated. Those results were then compared with the base case which incorporates the effects of low PO2 in the regions away from the vascular bundle, a result of the radial organization of tubules and vessels in the OM.

Concentration Profiles Under Well-Oxygenated Conditions

The synthesis of both NO and O2 is O2 dependent. Given that PO2 is low in the OM, the production of NO is likely to be severely limited by the availability of oxygen. As described in our previous study (5), the segregation of O2-rich long DVR within the core of the vascular bundle limits O2 reabsorption from these vessels, preserves O2 delivery to the inner medulla, and creates large radial PO2 gradients between regions. Region R1, which represents the bundle core, is well oxygenated, but in the surrounding regions, interstitial PO2 is predicted to be much lower, as illustrated in Fig. 2. At the mid-OS, interstitial PO2 is calculated as 56.3, 17.5, 9.7, and 9.0 mmHg, respectively, in R1, R2, R3, and R4; at the mid-IS, it is calculated as 49.7, 8.4, 3.9, and 9.7 mmHg, respectively. At the mid-IS, interstitial PO2 is lowest in R3, in part because of its largest population of mTAL, whose metabolic needs are high due to active NaCl reabsorption; see Fig. 1.)

To understand how low medullary PO2 affects NO, O2, and ONOO concentration profiles, in the first set of simulations we assumed that the generation rate of NO and O2 is independent of PO2. This would be the case if PO2 were very high and not rate limiting. In other words, the value of $K_{O2}$ in the NO generation rate was set to zero (Eq. 16), and the O2 generation rate was fixed at $O_{2}^{-}$, highPO2.

NO. In the OM, NO is generated within vascular endothelia and tubular epithelia. A fraction of that NO then diffuses from those cell layers toward the interstitium, and another fraction diffuses toward the vascular or tubular lumen. As water is reabsorbed from descending vessels and limbs into the interstitium and subsequently removed by AVR, a negligible portion of NO in the interstitium is also driven by convection into AVR. In every compartment, a small amount of NO reacts with O2 and ONOO, a much larger amount reacts with Hb; the rate of NO consumption by Hb (v3, v4) is ~10^3 times faster than that by O2 (v2), and ~10^6 times faster than that by O2 (v1).

As displayed in Fig. 3, predicted $C_{NO}$ values at the mid-IS are relatively homogeneous within each region, with the ex-

Table 2. Region parameters

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radii, μm</td>
<td>38.0/42.4</td>
<td>202/121</td>
<td>313/191</td>
<td>352/226</td>
</tr>
<tr>
<td>Interstitial areas, $\times 10^{-6}$ cm²</td>
<td>2.38/2.26</td>
<td>71.9/16.2</td>
<td>128.4/137</td>
<td>61.7/91.6</td>
</tr>
<tr>
<td>Fractional area available for diffusion, $A_{F_{R, R+1}}$</td>
<td>0.0524/0.0398</td>
<td>0.0702/0.120</td>
<td>0.0878/0.199</td>
<td>0.382/0.376</td>
</tr>
<tr>
<td>Perimeter of capillary RBC cytosol compartment, μm</td>
<td>0.073/1.10</td>
<td>32.2/191</td>
<td>32.4/253</td>
<td>3.78/42.1</td>
</tr>
<tr>
<td>Area of capillary RBC cytosol compartment, $\times 10^{-6}$ cm²</td>
<td>0.0054/0.0794</td>
<td>2.38/12.9</td>
<td>2.40/17.0</td>
<td>0.279/2.83</td>
</tr>
<tr>
<td>Perimeter of capillary endothelial compartment, μm</td>
<td>0.078/1.17</td>
<td>34.4/206</td>
<td>34.6/272</td>
<td>4.04/45.3</td>
</tr>
<tr>
<td>Area of capillary endothelial compartment, $\times 10^{-6}$ cm²</td>
<td>0.0008/0.0114</td>
<td>0.333/1.98</td>
<td>0.335/2.62</td>
<td>0.0390/0.437</td>
</tr>
</tbody>
</table>

The first value corresponds to the mid-OS, and the second to the mid-IS.
Table 4. **Endothelial and epithelial solute generation rates**

<table>
<thead>
<tr>
<th>Parameter Definition</th>
<th>Parameter Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal NO generation rate, ( G_{\text{NO max}} ), ( \mu \text{M} \cdot \text{s}^{-1} )</td>
<td>76.6</td>
</tr>
<tr>
<td>( \text{O}_2^- ) generation rate at high ( \text{PO}<em>2 ), ( G</em>{\text{O}_2^-}^{\text{highPo}_2} ), ( \mu \text{M} \cdot \text{s}^{-1} )</td>
<td>0.700</td>
</tr>
<tr>
<td>Area of vascular endothelium/tubular epithelium, ( \times 10^{-6} ) cm(^2)/vessel or tubule</td>
<td>All DVR: 0.377</td>
</tr>
<tr>
<td></td>
<td>LDV: 0.513/0.523</td>
</tr>
<tr>
<td></td>
<td>SDV: 0.513/0.551</td>
</tr>
<tr>
<td>Area of plasma/tubular lumen, ( \times 10^{-6} ) cm(^2)/vessel or tubule</td>
<td>All DVR: 0.881/0.513</td>
</tr>
<tr>
<td></td>
<td>LDV: 0.729/0.671</td>
</tr>
<tr>
<td></td>
<td>SDV: 0.542/0.444</td>
</tr>
<tr>
<td>Area of RBC compartment, ( \times 10^{-6} ) cm(^2)/vessel</td>
<td>LDV: 0.437/0.428</td>
</tr>
<tr>
<td></td>
<td>SDV: 0.437/0.399</td>
</tr>
</tbody>
</table>

DVR, descending vasa recta; LDV and SDV, long and short descending vasa recta, respectively; LAVa and LAVb and SAVa and SAVb, two populations of LAV and SAV, respectively; LDL and SDL, long and short descending limbs of Henle’s loop, respectively; LAL and SAL, long and short ascending limbs of Henle’s loop, respectively; CD, collecting ducts; N.A. not applicable. *When 2 values are given, the first corresponds to the mid-OS, the second to the mid-IS.
ments, and the former reaction is significantly faster than the latter (v₂ is 1–2 orders of magnitude greater than v₃).

In contrast with C₅₀₋, C₅₂₋ do not increase from the vascular bundle core outward. This is because the RBCs do not constitute a sink for O₂⁻: the dominant O₂⁻ scavenger is SOD, which is taken to be present in the same concentration everywhere (i.e., in all cells, plasma, and tubular fluid). Thus, contrary to NO, the net generation rate of O₂⁻ (i.e., production minus consumption) is positive in all vessels and tubules, as shown in Fig. 6. Given that the volumetric generation rate of O₂⁻ is highest in vasa recta and descending limbs (Table 4), C₅₂₋ varies in proportion with the fractional area occupied by vasa recta and descending limbs within each region. At the mid-OS, this fractional area is 43.0% in R₁, 6.4% in R₂, 13.4% in R₃, and 9.9% in R₄; thus interstitial C₅₂₋ decreases and increases accordingly (results not shown). At the mid-IS, the relative area occupied by vasa recta and descending limbs is 23.4% in R₁, 14.1% in R₂, 10.3% in R₃, and 4.0% in R₄; thus interstitial C₅₂₋ decreases monotonically from R₁ to R₄ (Fig. 5).

Even though the O₂⁻ volumetric generation rate is significantly larger in endothelium than in epithelia (Table 4), more O₂⁻ is generated in a given ascending limb than in an ascending vas rectum (34); note that the former is much wider than the latter (Table 1). This difference in surface area also means, however, that more O₂⁻ is consumed (mainly by SOD) in ascending limbs than in AVR. Hence, both the net generation rate and the concentration of O₂⁻ are lower in ascending limbs than in AVR (Figs. 5–6). The effect of surface area is such that, more generally, C₅₂₋ values are the highest in the endothelia of vasa recta and capillaries in each region. Within vessels, O₂⁻ concentration decreases from endothelium to the RBC, because O₂⁻ generated in the endothelium must first diffuse into the plasma and then into the RBCs. At the mid-IS, C₅₂₋ decreases by a factor of 5–10 from endothelium to plasma, and by a factor of 10–20 from plasma to RBC. The decrease in C₅₂₋ is sharper in AVR at the mid-OS, because these vessels are much wider at that level (27.0 μm in diameter) than at the mid-IS (13.5 μm in diameter). It is noteworthy that a larger endothelium or epithelium gives rise to a higher O₂⁻ generation rate as well as a larger area for diffusion out of the cellular layer. Our simulation results suggest that in general, these two competing factors result in higher O₂⁻ concentration in larger endothelium or epithelium. In the tubules, which are generally wider than the vasa recta, the epithelium-to-lumen C₅₂₋ ratio is higher, ranging from 15 to 200. Luminal C₅₂₋ is lowest in CDs, which have a large diameter (30.5 μm at the mid-OS, 24.7 μm at the mid-IS) and produce little O₂⁻. Long descending limbs are larger (their diameter is taken

---

**Table 5. Vessel and Tubule Permeability to NO, O₂⁻, and ONOO**

<table>
<thead>
<tr>
<th>Vessel or Tubule</th>
<th>*NO Permeability, cm/s</th>
<th>*O₂⁻ Permeability, cm/s</th>
<th>*ONOO Permeability, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long descending vasa recta</td>
<td>1.00</td>
<td>5.60 × 10⁻²</td>
<td>5.20 × 10⁻²</td>
</tr>
<tr>
<td>Short descending vasa recta</td>
<td>1.00</td>
<td>5.60 × 10⁻²</td>
<td>5.20 × 10⁻²</td>
</tr>
<tr>
<td>Long ascending vasa recta</td>
<td>2.44 × 10⁻²</td>
<td>5.60 × 10⁻²</td>
<td>5.20 × 10⁻²</td>
</tr>
<tr>
<td>Short ascending vasa recta</td>
<td>2.44 × 10⁻²</td>
<td>5.60 × 10⁻²</td>
<td>5.20 × 10⁻²</td>
</tr>
<tr>
<td>Long descending limbs</td>
<td>2.77/1.79</td>
<td>5.09 × 10⁻²</td>
<td>4.72 × 10⁻²</td>
</tr>
<tr>
<td>Short descending limbs</td>
<td>2.77/2.53</td>
<td>5.09 × 10⁻²</td>
<td>4.72 × 10⁻²</td>
</tr>
<tr>
<td>Long ascending limbs</td>
<td>2.44 × 10⁻³</td>
<td>7.00 × 10⁻³</td>
<td>6.50 × 10⁻³</td>
</tr>
<tr>
<td>Short ascending limbs</td>
<td>2.44 × 10⁻²</td>
<td>7.00 × 10⁻³</td>
<td>6.50 × 10⁻³</td>
</tr>
<tr>
<td>Collecting ducts</td>
<td>2.44 × 10⁻²</td>
<td>6.22 × 10⁻³</td>
<td>5.78 × 10⁻³</td>
</tr>
</tbody>
</table>

*Permeabilities are given for the cell-interstitial and lumen-cell interfaces, which are taken to be equal. †The permeability of descending limbs varies between the mid-OS (first value) and the mid-IS (second value) because aquaporin-1 (AQP1) expression also varies.

---

**Fig. 2. Oxygen tension (PO₂) profiles at the mid-IS in the interstitium, vasa recta, capillaries, and tubules in each region. Each tubule or vas rectum is assigned to the region with which it is in contact for 50% or more in the IS. In vasa recta, PO₂ values in red blood cells (RBCs), plasma, and endothelium are displayed separately. In capillaries (denoted “cRBC”), PO₂ values in the RBC cytosol and surrounding endothelium are also distinguished. Similarly, in tubules, PO₂ values in lumen and epithelium are shown separately. The term “cellular layer” denotes the endothelium in vasa recta and capillaries, and the epithelium in tubules. Data are taken from Ref. 4.**
to vary from 80.0 to 18.6 μm), but they produce significantly more O₂⁻ (Table 4).

ONOO. ONOO is formed by the reaction between NO and O₂⁻. As shown in Fig. 7, ONOO concentrations (CONO₂) are predicted to be in the 0.01–2 nM range. Within each region, they are highest in vascular endothelia and tubular epithelia, that is, the compartments where NO and O₂⁻ are produced and where these species are the most abundant. The most potent ONOO scavengers, Prx2 and HbO₂, are in the RBCs. Thus ONOO concentrations decrease significantly more from plasma to RBC than from endothelium to plasma. Prx2 and HbO₂ are absent from tubules; the ONOO concentration gradient from epithelium to tubular lumen is therefore comparable to that from endothelium to plasma, albeit larger, because most tubules are wider than vessels.

As described above, CNO increases from the vascular bundle core toward the outer regions, at both the mid-OS and the mid-IS. In contrast, at the mid-IS, interstitial CNO₂⁻ drops sharply between R1 and R2 and then varies little between R2, R3, and R4. At that level, the product of CNO and CNO₂⁻ is the lowest in R2, and therefore so is CONO₂ (results not shown). At the mid-IS, the product of CNO and CNO₂⁻ decreases monotonically from R1 to R4, and therefore so does CONO₂ (Fig. 7).

Base-Case Concentration Profiles

To study the effects of low PO₂ on the distribution of NO, O₂⁻, and ONOO in the OM, we accounted for the effects of medullary hypoxia on the NO and O₂⁻ generation rates. In these simulations, the PO₂ value at which NO synthesis is reduced two-fold (K_{NO_{O2}}, see Eq. 16) was taken as 38 mmHg (50), i.e., a midrange value. Given the conflicting findings regarding the effects of hypoxia on O₂⁻ synthesis, as described in detail above in MODEL DESCRIPTION, we examined several scenarios: the O₂⁻ generation rate was taken to either decrease with decreasing PO₂ (case A), to remain independent of PO₂ (case B, or to increase with decreasing PO₂ (case C).

NO concentrations. As described in the companion study (15), under physiological conditions O₂⁻ has a small impact on NO bioavailability. Hence, predicted NO concentrations differ by <2% in cases A, B, and C, and results are shown for case B.
In the core of the vascular bundle, PO$_2$ hovers around 50 mmHg (Fig. 2), but at the bundle periphery, interstitial PO$_2$ is predicted to be lower than 18 mmHg at the mid-OS, and 10 mmHg at the mid-IS. Thus the extent to which the generation rate of NO is reduced by limited O$_2$ availability is much greater in the outer regions (R2–R4) than in R1. As a consequence, base-case NO concentrations are predicted to be maximal in R1, in contrast to the trends obtained under well-oxygenated conditions. At the mid-IS, C$_{NO}$ is reduced by a factor of ~2 in R1, and ~5–8 to in R3-R4, relative to the well-oxygenated case (Fig. 8). Interstitial C$_{NO}$ range from 104 nM in R1 (vs. 213 with $K_{O2}^{NO}$) to 42 nM in R3 (vs. 343 with $K_{O2}^{NO}$). The highest NO concentrations (~180 nM) are still found in the endothelium of the widest, and least-permeable, vessels in the vascular bundle, that is, the long ascending vasa recta in R1 (Fig. 8). NO fluxes are largest at the endothelium-plasma and plasma-RBC interfaces of DVR and AVR in R1.

$O_2^-$ concentrations. Hypoxia reduces the rate of $O_2^-$ consumption by decreasing the generation rate (and thus the concentration) of one of its scavengers, NO. If we assume that low medullary PO$_2$ also reduces the rate of $O_2^-$ synthesis (case A), then which of these competing effects prevails depends on the region. In the vascular bundle core they almost balance each other, so that in R1, basal $C_{O2^-}$ are close to the values predicted for the well-oxygenated case (Fig. 9). In R2 and R3, where O$_2$ is the scarcest, the reduction in $O_2^-$ synthesis predominates, and basal $C_{O2^-}$ are significantly lower than in the well-oxygenated case. For instance, at the mid-IS, interstitial $C_{O2^-}$ is 26 and 46% lower in R2 and R3, respectively (relative to the well-oxygenated case). In R4, where PO$_2$ is slightly higher than in R2 and R3 at the mid-IS, the reduction in vascular and tubular $O_2^-$ synthesis is not as significant as in the latter regions, but $C_{O2^-}$ is generally lower than in the well-oxygenated case.

Assuming that $O_2^-$ synthesis is independent of O$_2$ levels (case B), low medullary PO$_2$ only affects the consumption of $O_2^-$ by NO and leads to a $C_{O2^-}$ increase throughout the medulla, as shown in Fig. 9. This increase is even greater in case C, which assumes that hypoxia stimulates $O_2^-$ synthesis. Under these conditions, low medullary PO$_2$ raises $O_2^-$ levels...
under the combined effect of higher production and lower consumption. At the mid-IS, $O_2^{-}$ concentrations are two- to fivefold greater than under well-oxygenated conditions. The increase is more pronounced in the peripheral regions (R2–R4) where the $O_2^{-}$-induced inhibition of NO synthesis is more significant, and where $O_2^{-}$ scavenging by NO therefore decreases more (Fig. 9). As in the well-oxygenated case,Interstitial $O_2^{-}$ is the highest in R1, where the fractional area occupied by vasa recta is the highest.

Predicted $O_2^{-}$ concentrations range from 0.1 to 170 pM in case A, from 0.3 to 240 pM in case B, and from 0.5 to 360 pM in case C. In all cases, $O_2^{-}$ fluxes are largest across AVR endothelia.

**ONOO concentrations.** The rate of ONOO synthesis is proportional to the NO and $O_2^{-}$ concentration product ($\text{Eq. 21}$) and therefore varies accordingly. For instance, at the mid-IS, the product of CNO and $O_2^{-}$ in the endothelium of the long DVR located in R1 is 34.2 in the well-oxygenated case, and 16.2 in case A (that is, 2.1 times lower). The DVR endothelial concentration of ONOO in those vessels follows the same trend: it is 1.25 nM in the well-oxygenated case, and 0.63 nM in case A (that is, 2.0 times lower).

ONOO concentrations are the lowest in case A because the latter assumes that generation rates of both NO and $O_2^{-}$ are reduced by hypoxia. In case A, C ONOO is about 2-fold lower in R1 and 7- to 20-fold lower in R2–R4, relative to the well-oxygenated case (Fig. 10). Predicted ONOO concentrations then range from 1 to 730 pM. In case B, C $O_2^{-}$ and therefore C ONOO are higher than in case A; the latter varies between 2 and 960 pM.

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**Fig. 7.** Peroxynitrite (ONOO) concentrations ($C_{\text{ONOO}}$) at the mid-IS in the interstitium, vasa recta, capillaries, and tubules in each region under well-oxygenated conditions.

**Fig. 8.** Effect of low medullary $P_O_2$ on CNO at the mid-IS in interstitium, vasa recta, capillaries, and tubules. In this figure and Figs. 9 and 10, concentrations in vessels and tubules are those of the endothelium/epithelium. In addition, the “high $P_O_2$” case corresponds to well-oxygenated conditions ($K_{O_2}^{\text{NO}} = 0$), whereas “low $P_O_2$” refers to the base case ($K_{O_2}^{\text{NO}} = 38 \text{ mmHg}$). Results for cases A, B, and C cannot be distinguished.
In case C, as described above, medullary hypoxia reduces $C_{NO}$ and increases $C_{O2^-}$ by a factor of ~2 in the vascular bundle core. Thus ONOO concentrations in R1 are not much different than in the well-oxygenated case. In the peripheral regions, however, $C_{ONOO}$ remains significantly lower than in the well-oxygenated case, since the hypoxia-induced decrease in $C_{NO}$ is much greater than the hypoxia-induced increase in $C_{O2^-}$ in those regions. Hence, $C_{ONOO}$ is about twofold lower in R2–R4, relative to the well-oxygenated case (Fig. 10). Predicted ONOO concentrations then range from 3 pM to 1.4 nM. In all cases, ONOO fluxes are largest at the endothelium-to-plasma interface in long ascending vasa recta in R1.

**Effects of $K_{O2}^{NO}$ Variations**

Given that the range of reported $K_{O2}^{NO}$ values is wide (see above), we then examined the effect of varying the latter parameter in a set of parameter studies. To facilitate result interpretation, the $O2^-$ generation rate was kept constant in those studies; i.e., it was fixed as in case B.

Assuming a lower bound value of 5 mmHg for $K_{O2}^{NO}$, the rate-limiting effects of $O2^-$ on the NO generation rate are less considerable than in the base case, but they are significant nonetheless, as illustrated in Fig. 11A. Since the $O2^-$-mediated decrease in $G_{NO}$ is smaller when $K_{O2}^{NO}$ is taken as 5 mmHg than in the base case, NO concentrations are conversely higher.
Relative to the base case, the predicted $C_{NO}$ is about twofold higher in R1, and three- to fourfold higher in R2−R4. At the mid-IS, interstitial, endothelial, and epithelial $C_{NO}$ are remarkably homogeneous across all regions, ranging from 167 to 184 nM (Fig. 11A).

Conversely, if $K_{O2}^{NO}$ is taken as 60 mmHg, i.e., an upper range value (2), $G_{NO}$ drops even further than in the base case and $C_{NO}$ are significantly reduced. In every compartment except for AVR and capillary endothelium, they are predicted to remain below 90 nM at the mid-OS, and 82 nM at the mid-IS. In the interstitium, $C_{NO}$ ranges from 29 to 82 nM at the mid-IS (Fig. 11A).

Since $K_{O2}^{NO}$ has a large impact on medullary $C_{NO}$, it also has a significant effect on $O_2$ consumption, and therefore on $C_{O2−}$. If $K_{O2}^{NO}$ is lowered to 5 mmHg, $C_{NO}$ rises relative to the base case (where $K_{O2}^{NO} = 38$ mmHg), thereby increasing $O_2$ scavenging by NO, and reducing $C_{O2−}$. Given that $P_{O2}$ is less rate limiting in the vascular bundle, concentration variations are also smaller in that region. Relative to the base case, $C_{O2−}$ is $\sim$20–30% lower in R1, and 30–50% lower in R2−R4. Conversely, if $K_{O2}^{NO}$ is taken as 60 mmHg, $C_{O2−}$ increases by $\sim$10% relative to the base case (Fig. 11B).

The lower $K_{O2}^{NO}$, the higher $C_{NO}$, and the higher the rate of ONOO formation. Thus $C_{ONOO}$ increases significantly as $K_{O2}^{NO}$ is decreased. If $K_{O2}^{NO}$ is taken as 5 mmHg, $C_{ONOO}$ is 40% higher in R1, and 110–180% higher in R2−R4, relative to the base. The peak interstitial $C_{ONOO}$ is then 1.18 nM, vs. 0.85 nM in the base case. If $K_{O2}^{NO}$ is increased to 60 mmHg instead, $C_{ONOO}$ is reduced by $\sim$15% in R1, and $\sim$30% in R2−R4, relative to the base case. The peak interstitial $C_{ONOO}$ is then 0.72 nM (Fig. 11C).

**DISCUSSION**

We have developed a mathematical model of a cross section of the rat OM to simulate the interactions between NO and $O_2$−. The model accounts for the relative positions of the tubules and vessels in the OM by representing four concentric regions, centered on a vascular bundle (Fig. 1). The model also takes into account medullary $O_2$ distribution and radial convection by incorporating $P_{O2}$ and volume flux data from a three-dimensional model of oxygen transport in the rat OM that we developed previously (4, 5).

In the current study, we examined the impact of the three-dimensional architecture of the rat OM on the distribution of NO, $O_2$−, and ONOO. The companion study (15) focuses on the importance of tubulovascular cross talk and the interactions between NO and $O_2$−. The radial organization of the rat OM around vascular bundles has two implications for NO, $O_2$−, and ONOO. First, the sequestration of $O_2$-rich DVR within the vascular bundles results in large radial $P_{O2}$ gradients from the vascular bundle core (R1) toward the outer regions (R2−R4) (5). Those $P_{O2}$ gradients, taken in isolation, mean that the generation of NO is slower away from the bundles. Second, the vascular bundles have the highest density of RBCs, which carry the most potent NO scavenger, namely Hb.

Our present results suggest that the first of these two competing effects predominates: the steep radial $P_{O2}$ gradients generate similar radial NO and ONOO concentration gradients. As displayed in Fig. 8, the base-case concentration of NO is predicted to be the highest in the bundle core, where $O_2$ is relatively plentiful, and where generation rates are therefore significantly larger than at the bundle periphery. If the synthesis of NO were not limited by $O_2$ availability, $C_{NO}$ would be the lowest in R1, owing to the high density of vasa recta in that region combined with the very fast rate of NO consumption by Hb and HbO2 in RBCs (Fig. 8). In contrast, interstitial $C_{O2−}$ is highest in R1 under all conditions, because the volumetric rate of $O_2$− generation is the largest in vasa recta, the density of which is maximal in R1, while there are no $O_2$− potent scavenger.
scavengers in RBCs (Fig. 9). Thus ONOO would be more homogeneously distributed across the four regions in the absence of O2 rate-limiting effects (Fig. 10).

Comparison With a Previous Modeling Study

Our previous model of cross-sectional NO transport, referred to as the ZE model (53), did not include the effects of medullary hypoxia on NO generation rates. Thus it predicted that the average interstitial CNO is highest in R2, where the density of descending limbs, which represent the most significant epithelial source of NO, is the highest. However, the current study suggests that the O2 dependence of NO and O2− synthesis has a large impact on medullary concentration profiles, and that it leads to a sharp CNO decrease between the vascular bundle core (R1) and the peripheral regions (R2–R4), as described above.

In addition, the ZE model did not explicitly consider the generation, transport, and consumption of C2O− and CONO2. Instead, the concentration of C2O− was fixed at 0.25 nM in the interstitium, 0.10 nM in endothelium, and 0.05 in plasma. The basal C2O− values predicted by the current model are lower in the interstitium (ranging from 0.01 to 0.21 nM) but comparable in plasma. As such, the ZE model may have overestimated the effect of a 10-fold increase in O2− levels on NO, as discussed in the companion study (15).

Finally, the ZE model did not incorporate radial convection. We examined the impact of radial convection in the current model by setting all the transmembrane water fluxes to zero: the effect on the predicted concentration profiles is negligible (<0.1%), because Pécelt numbers (i.e., the ratio of convective-to-diffusive fluxes; Eq. 10) are lower than 10−2 everywhere.

Comparison with Experimental Findings

Measurements of physiological CNO in the OM range from 100 (56) to 800 nM (44, 45). In this study, we chose the base-case NO generation rate so that theoretical NO concentrations would be comparable to the lower bound measurements, as discussed in more detail in the companion study (15). Thus, in the base case, interstitial CNO are predicted to range from 63 to 112 nM at the mid-OS, and from 42 to 104 nM at the mid-IS (Fig. 8).

To the best of our knowledge, there have been no direct measurements of O2− and ONOO concentrations in the renal medulla. Under physiological (i.e., low PO2) conditions, our model yields medullary C2O− on the order of 10–200 pM in the interstitium and ~1–10 pM within RBCs. Buerk et al. (1) developed a model of NO and O2− transport in and around a small arteriole. They assumed a lower NO generation rate than the one used here, and their model yields lower CNO (~20 nM in the perivascular tissue surrounding the small arteriole), and therefore slightly higher C2O− (100–250 pM in tissue), than the ones predicted in this study. Our C2O− estimates are also comparable to, if slightly higher than, the 50–50 pM range estimated by Ferrer-Sueta and Sadi (18) in their review of the chemical biology of ONOO.

Our model predicts that interstitial concentrations of total ONOO (ONOO− and ONOOH) at the mid-IS range from ~0.05 to 1 nM under physiological conditions. Within RBCs, the predicted CONO2 varies between 5 and 30 pM in the AVR located in the outermost regions, and between 20 and 130 pM in the vascular bundle vessels. These values are consistent with the range predicted by Ferrer-Sueta and Sadi (18), who suggested that CONOO should not exceed 2–3 nM under basal conditions, and could plummet to <25 pM in erythrocytes.

Effects of NO on Medullary Blood Flow Distribution

DVR are surrounded by pericytes, smooth muscle cells that impart contractile properties to these vessels. Solute concentrations in pericytes, which are not explicitly considered in this study, should be close to those in the endothelium. In the current base case, the predicted endothelial CNO is 72% higher in long DVR than in short DVR at the mid-OS (110 vs. 64 nM), and 66% higher at the mid-IS (103 vs. 62 nM). Long DVR are located at the center of the vascular bundle and are destined to the inner medulla, whereas short DVR peel off from the bundle periphery and supply the OM. Given the vasodilatory properties of NO, the long DVR-to-short DVR pericite CNO ratio is expected to modulate the distribution of medullary blood flow between the inner medulla and OM (41). As we described previously (53), the experimental results of Zhang et al. (55) and Kakoki et al. (27) suggest that CNO variations on the order of 10 nM may affect blood flow. Our results therefore suggest that the difference in endothelial CNO between long DVR and short DVR preferentially increases blood flow to the inner medulla under basal conditions.

It should be noted that our previous modeling study had predicted higher NO concentrations in short DVR than in long DVR, but that model had assumed fixed, O2−-independent NO generation rates (53). Under similar conditions (i.e., when O2 is plentiful), the current model also predicts that CNO is higher in short DVR than in long DVR (Fig. 3).

Model Limitations

The model presented here only represents a slice of the rat OM and does not describe countercurrent flows along the corticomediullary axis. It is limited to the transport of NO, O2−, and ONOO and cannot predict the effects of these radicals on NaCl reabsorption across the mTAL and OM concentrating capability, and on blood flow. The transmembrane O2− fluxes do not take into account the electrical charge of the species. In addition, because only steady-state solutions were sought, the model does not capture the effects of transient events. At each systole, vascular endothelial cells release a puff of NO (20). The impact of these puffs beyond local areas in the endothelial cell cytoplasm remains unclear. As NO diffuses from multiple cells into the interstitium, the integrated effects of many different cells will be superimposed on the rhythmic release of NO from the endothelium. The NO puffs may not generate a standing wave unless they are synchronized. In short, it is possible that medullary CNO gradients vary with the cardiac cycle, but there are not enough data as yet to investigate this issue.

Nevertheless, the model constitutes a convenient tool to examine the impact of kinetic and transport parameters on the distribution of NO, O2−, and ONOO in the OM. In addition, it explicitly accounts for the endothelial and epithelial layers, whereas three-dimensional models of water and solute transport in the medulla represent vascular and capillary walls as a single barrier. Since NO and O2− are generated within vascular endothelia and tubular epithelia, explicit consideration of these cellular layers yields more accurate results.
The current study shows how the large radial PO2 gradients in the rat OM, which arise from the specific three-dimensional architecture of the rat OM, affect the distribution of NO, O2\(^{-}\), and ONOO. The reciprocal effects of NO on medullary PO2 may be significant as well. As a vasodilator, NO acts to increase local blood flow and O2 supply. NO also reduces tubular O2 demand by inhibiting active NaCl reabsorption across mTALs (23). Moreover, NO inhibits mitochondrial O2 utilization by competing with O2 for binding to mitochondrial cytochrome c oxidase. Since the CNO at which tissue O2 consumption is inhibited by half (IC\(_{50}\)) is on the order of 10–100 nM, and since perivascular NO concentrations are generally higher than IC\(_{50}\) values, cellular respiration is likely to be significantly inhibited under physiological conditions (7). As noted above, O2\(^{-}\) exerts opposite effects: it enhances mTAL Na\(^{+}\) reabsorption and acts to reduce medullary blood flow. There is also evidence that ONOO\(^{-}\), in turn generate steep radial CNO gradients, since specific three-dimensional organization of tubules and vessels bundle core toward the outer regions, which stem from the OM, under basal conditions.

In summary, our model of NO, O2\(^{-}\), and ONOO transport in the rat OM suggests that the large PO2 gradients from the bundle core toward the outer regions, which stem from the specific three-dimensional organization of tubules and vessels in the OM, in turn generate steep radial CNO gradients, since NO synthesis is O2\(^{-}\) dependent. The resulting difference in endothelial CNO between long DVR and short DVR is likely to increase blood flow to the inner medulla, to the detriment of the OM, under basal conditions.

### Glossary

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>( A_i )</td>
<td>Cross-sectional area of compartment i</td>
</tr>
<tr>
<td>( A_{F,R,R'} )</td>
<td>Fraction of the R-R' interface available for interstitial diffusion</td>
</tr>
<tr>
<td>AVR</td>
<td>Ascending vasa recta</td>
</tr>
<tr>
<td>( C_{NO}, C_{O2−}, C_{ONOO} )</td>
<td>Concentration of NO, O2(^{-}), and ONOO</td>
</tr>
<tr>
<td>( C_i^\text{v} )</td>
<td>Concentration of solute k in compartment i</td>
</tr>
<tr>
<td>( D_k )</td>
<td>Diffusivity of solute k in dilute solution</td>
</tr>
<tr>
<td>( d_{R,R'} )</td>
<td>Distance between the midpoints of regions R and R'</td>
</tr>
<tr>
<td>DVR</td>
<td>Descending vasa recta</td>
</tr>
<tr>
<td>( f_{\text{ion}} )</td>
<td>Fraction of total peroxynitrite that is present in ionic form</td>
</tr>
<tr>
<td>( G_{NO}, G_{O2−} )</td>
<td>Volumetric rate of NO and O2(^{-}) generation</td>
</tr>
<tr>
<td>( G_{NO, \text{max}} )</td>
<td>Maximal volumetric rate of NO generation</td>
</tr>
<tr>
<td>IS</td>
<td>Inner stripe</td>
</tr>
<tr>
<td>( f_{k,i}^\text{j} )</td>
<td>Flux of solute k from compartment i to compartment j</td>
</tr>
<tr>
<td>( f_{\text{tot}R} )</td>
<td>Total flux of solute k into region R</td>
</tr>
<tr>
<td>( f_{i,k}^\text{v,j} )</td>
<td>Flux of volume from compartment i to compartment j</td>
</tr>
<tr>
<td>( K_{NO}^\text{O2} )</td>
<td>Oxygen tension at half the maximal rate of NO generation</td>
</tr>
<tr>
<td>OM</td>
<td>Outer medulla</td>
</tr>
<tr>
<td>OS</td>
<td>Outer stripe</td>
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### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

### REFERENCES


