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Impact of nitric oxide-mediated vasodilation on outer medullary NaCl transport and oxygenation

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Edwards A, Layton AT. Impact of nitric oxide-mediated vasodilation on outer medullary NaCl transport and oxygenation. Am J Physiol Renal Physiol 303: F907–F917, 2012. First published July 11, 2012; doi:10.1152/ajprenal.00055.2012.—The present study aimed to elucidate the reciprocal interactions between oxygen (O₂), nitric oxide (NO), and superoxide (O₂⁻) and their effects on vascular and tubular function in the outer medulla. We expanded our region-based model of transport in the rat outer medulla (Edwards A, Layton AT. Am J Physiol Renal Physiol 301: F979–F996, 2011) to incorporate the effects of NO on descending vasa recta (DVR) diameter and blood flow. Our model predicts that the segregation of long DVR in the center of vascular bundles, away from tubular segments, gives rise to large radial NO concentration gradients that in turn result in differential regulation of vasoreactivity in short and long DVR. The relative isolation of long DVR shields them from changes in the rate of NaCl reabsorption, and hence from changes in O₂ requirements, by medullary thick ascending limbs (mTALs), thereby preserving O₂ delivery to the inner medulla. The model also predicts that O₂⁻ can sufficiently decrease the bioavailability of NO in the interbundle region to affect the diameter of short DVR, suggesting that the experimentally observed effects of O₂⁻ on medullary blood flow may be at least partly mediated by NO. In addition, our results indicate that the tubulovascular cross talk of NO, that is, the diffusion of NO produced by mTAL epithelia toward adjacent DVR, helps to maintain blood flow and O₂ supply to the interbundle region even under basal conditions. NO also acts to preserve local O₂ availability by inhibiting the rate of active Na⁺ transport, thereby reducing the O₂⁻ requirements of mTALs. The dual regulation by NO of oxygen supply and demand is predicted to significantly attenuate the hypoxic effects of angiotensin II.

model: kidney; oxygen; superoxide; tubulovascular cross talk

NITRIC OXIDE (NO) AND SUPEROXIDE (O₂⁻) exert opposite effects on renal vascular and tubular function. Inhibition of NO synthesis leads to a reduction in medullary blood flow (MBF), salt retention, and hypertension (27). The effects of NO on the renal medulla are mediated by several pathways: NO exerts a strong vasodilatory influence on descending vasa recta (DVR) (32), and it inhibits NaCl reabsorption in the medullary thick ascending limb (mTAL) by reducing the activity of the Na⁺−K⁺−2Cl⁻ cotransporter (30). In contrast, O₂⁻ stimulates NaCl transport across mTALs and favors reductions in MBF (29, 11). In addition, NO and O₂⁻ diminish each other’s bioavailability, as they react together to form peroxynitrite. A recent study also suggests that NO reduces flow-stimulated increases in O₂⁻ production via the cGMP/PKG pathway, independently of its scavenging effects on O₂⁻ (18). The importance of NO-O₂⁻ interactions is underscored by accumulating evidence that the balance between NO and O₂⁻ is one of the key mechanisms for the development of salt-sensitive hypertension (26).

Adding to the complexity of these interactions is the role of oxygen (O₂). O₂ is a substrate for the formation of each NO and O₂⁻, yet some studies have suggested that hypoxia enhances medullary NO levels (16) and O₂⁻ production (22). Moreover, the availability of O₂ depends on the balance between O₂ supply, itself a function of blood flow, and O₂ demand, which varies with the rate of active Na⁺ transport. To better understand the three-way interactions between O₂, NO, and O₂⁻, and how they impact tubular and vascular function, we recently developed a region-based model of O₂, NO, and O₂⁻ transport in the outer medulla (OM) of the rat (14). Our results suggested that NaCl transport and the concentrating capacity of the OM are substantially modulated by basal and pathological levels of NO and O₂⁻. This previous model, however, did not account for DVR vasoreactivity and subsequent changes in MBF and O₂ supply. It therefore lacked an important component of the feedback loops involving O₂, NO, and O₂⁻.

The model described herein incorporates the effects of NO on DVR diameter and DVR flow. It is first used to determine whether O₂⁻ -induced vasoconstriction may be in part mediated by NO. The second part of our study centers on the impact of tubular NO synthesis. We examine in particular the hypothesis that diffusion of vasoactive agents such as NO from adjacent tubules to vasa recta plays an important role in protecting the medulla from hypoxic injury, by modulating blood flow and O₂ supply in response to variations in mTAL transport and metabolical requirements (28).

MODEL DESCRIPTION

Our “region-based” model accounts for the three-dimensional architecture of the rat OM by distinguishing four concentric regions, as originally proposed by Layton and Layton (21): the innermost region (R1) represents the central vascular bundle, where all the long DVR and a third of the long ascending vasa recta (AVR) are sequestered; the surrounding region (R2) represents the immediate periphery of the bundle and encompasses all short DVR (those that turn within the OM) and the remaining long AVR; the neighboring region (R3) contains most mTALs, both long and short, and some short AVR; and the region most distant from the vascular...
bundle (R4) includes all the collecting ducts and the remaining short AVR (see Fig. 1 in Ref. 14).

The model determines flows and solute concentrations as a function of OM depth in the interstitium, the loops of Henle, collecting ducts, vasa recta, and capillaries. Blood flow in vasa recta is divided into two compartments, plasma and red blood cells (RBCs). The solutes explicitly considered in the model are NaCl, urea, deoxy- and oxy-hemoglobin (Hb and HbO2), O2, NO, HbNO, and O2. Detailed transport equations can be found in our previous studies (6, 14). We focus here on the new feature of our model, namely, NO-induced vasodilation of long (LDV) and short (SDV) DVR. We also briefly summarize the equations describing active Na⁺ transport and O2 consumption in the OM.

**NO-Induced Changes in DVR Diameter**

The relationship between NO levels and DVR diameter is complex. The DVR endothelium is surrounded by pericytes, vascular smooth muscle cells that impart vasoconstrictive properties to the vessels. The vasodilatory effects of NO appear to be mostly mediated by the cGMP/PKG pathway, which acts to reduce intracellular Ca²⁺ and the proportion of phosphorylated myosin light chains within pericytes (13). Moreover, vascular diameter is regulated by many other vasoactive agents, such as prostaglandins and endothelins. In light of this complexity, we opt for a simple, semiempirical approach. We assume that the local DVR radius (R_{DVR}) depends on local NO concentration according to

\[
\frac{R_{DVR}(x)}{R^*} = \frac{A[C_{NO}^{DVR}(x)/C_{NO}^{*}]}{(A - 1) + [C_{NO}^{DVR}(x)/C_{NO}^{*}]} \quad \text{DVR = LDV, SDV}
\]

(1)

where \(x\) denotes the position on the corticomedullary axis, \(C_{NO}^{DVR}\) is the NO concentration in DVR plasma (more generally, \(C_i\) is the concentration of solute \(i\) in compartment \(j\)), \(A\) is a constant to be determined, and the superscript * indicates reference values. The reference radius is taken as 5.5 \(\mu\text{m}\), which is the average interstitial NO concentration at the outer-inner medullary junction that we predicted in our previous base case (14).

The study of Kakoki et al. (20) relates changes in interstitial NO concentrations in the inner medulla (at a depth of 5.5 mm, that is, close to the junction between the outer and inner medulla) to changes in MBF. To relate, in turn, changes in MBF to variations in vessel radius, we use Poiseuille’s law

\[
\frac{dP}{dx} = -\frac{8\mu}{\pi} \left( \frac{F_{DVR}^{DVR}(x)}{R_{DVR}^{DVR}(x)} \right) \quad \text{DVR = LDV, SDV}
\]

(2)

where \(P\) is the luminal hydraulic pressure, \(F_{DVR}^{DVR}\) is the single-vessel blood flow, and \(\mu\) is the fluid viscosity. Specifically, we assume that MBF varies in proportion to \(R_{DVR}^{DVR}\). Kakoki et al. (20) found that different doses of NOS inhibitors respectively reduced NO levels by 32 and 66%, and MBF by 32 and 40%; with our assumptions, the latter correspond to 10 and 12% reductions in \(R_{DVR}^{DVR}\). A best fit of Eq. 1 to these data yields \(A = 1.0962\). The postulated dependence of \(R_{DVR}^{DVR}\) on \(C_{NO}^{DVR}\) is illustrated in Fig. 1. We assume that DVR constrict along their entire OM length, since some vessels exhibit pericytes all the way to the papillary tip (33). However, it is possible that only the upper parts of OM DVR are capable of vasoconstriction. Our qualitative conclusions would nevertheless remain unaffected.

Blood flow is calculated as follows. The pressure in the efferent arteriole (denoted P_E) and that in the renal vein (denoted P_V) are respectively set to 20 and 3 mmHg (4, 10, 19). Each DVR is connected in series to a fixed downstream resistance (\(\Gamma_{down}\)), the value of which depends on the DVR length and localization. Blood flow at the DVR inlet (i.e., at the corticomedullary junction) is computed by an iterative process, so that the sum of the pressure drop across the DVR (\(\Delta P_{DVR}\)) and that across its downstream resistance equals (\(P_E - P_V\))

\[
P_E - P_V = \Delta P_{DVR} + F_{DVR}^{DVR}(L_{DVR})\Gamma_{down}
\]

(3)

where \(L_{DVR}\) is the vessel length, and \(\Delta P_{DVR}\) is given by

\[
\Delta P_{DVR} = \frac{8\mu}{\pi} \int_0^{L_{DVR}} \frac{F_{DVR}^{DVR}(s)}{R_{DVR}^{DVR}(s)} ds
\]

(4)

Note that blood flow variations along DVR are calculated based upon the rate of water reabsorption (see Eqs. 3 and 4 in Ref. 14). The downstream resistance \(\Gamma_{down}\) of each DVR is chosen so that under baseline conditions, DVR blood flow at the inlet is 8 nl/min; \(\Gamma_{down}\) then remains fixed in all other simulations. The viscosity of blood flowing through vasa recta is low, not much higher than that of plasma (34); we assume \(\mu = 2\) cp. As an illustration, the pressure drop across a DVR that extends along the entire OM length would be \(11.7\) mmHg if its blood flow remained constant at 8 nl/min and its radius fixed at 5.5 \(\mu\text{m}\). The pressure drop across the downstream resistance would then equal (20–3) – 11.7 = 5.3 mmHg. Base-case flow and pressure profiles in long and short DVR are shown in Fig. 2.

The model represents the vasoactive effects of NO but assumes that the DVR radius is independent of the transmural-pressure gradient. This simplifying hypothesis allows us to avoid computing local fluid pressure along the DVR and leads to substantial savings in computational cost. While elevation of luminal pressure has been shown to progressively dilate DVR ex vivo owing to the absence of a myogenic response (38), according to our calculations the effects of pressure on the radius in the scenarios simulated below are small compared with those of NO. Whereas the pressure drops from \(20\) mmHg in the efferent arteriole to \(3\) mmHg in the renal vein, pressure variations at a given medullary level are estimated to be <0.3 mmHg.

**Fig. 1. Postulated dependence of descending vasa recta (DVR) radius on nitric oxide (NO) concentration (\(C_{NO}\)) in DVR plasma, as described by Eq. 1. The radius of the vessel cannot fall below that of a single erythrocyte (−4 \(\mu\text{m}\)).**
over. We posit that in the complete absence of O₂, the anaerobic pathway produces enough energy to sustain a transport rate that is a fraction \( \lambda \) (taken as 0.50) of the maximum rate when O₂ supply is abundant, so that

\[
f(P^\text{mTAL}_\text{O}_2) = \begin{cases} 
1 & P^\text{mTAL}_\text{O}_2 \geq P_c \vspace{1mm} \\
\lambda + (1 - \lambda)(P^\text{mTAL}_\text{O}_2/P_c) & P^\text{mTAL}_\text{O}_2 < P_c
\end{cases}
\]  

(6)

With these assumptions, \( f(P^\text{mTAL}_\text{O}_2) \) varies between 0.50 and 1. The function \( g(C^\text{mTAL}_\text{NO}) \) accounts for the inhibitory effect of NO on \( \Psi^\text{active}_\text{mTAL}, \text{Na} \) and is specified as (14)

\[
g(C^\text{mTAL}_\text{NO}) = 1 - \frac{C^\text{mTAL}_\text{NO}}{\beta + C^\text{mTAL}_\text{NO}}
\]  

(7)

where \( \beta \) is estimated as 46.9 nM. Similarly, the function \( h(C^\text{mTAL}_{O_2^{-}}) \) represents O₂⁻-induced stimulation of mTAL NaCl transport, and is chosen as (14)

\[
h(C^\text{mTAL}_{O_2^{-}}) = 0.7 + 0.6 \left( \frac{C^\text{mTAL}_\text{NO}}{C^\text{mTAL}_\text{NO} + C^\text{mTAL}_{O_2^{-}}} \right)
\]  

(8)

where the reference value \( C^\star_{O_2^{-}} \) equals 20 PM in scenario A and 350 PM in scenario B (see below), so that \( h(C^\text{mTAL}_{O_2^{-}}) \) equals 1 under baseline conditions.

The overall rate at which Na⁺ is reabsorbed along mTALs, denoted \( I^\text{TNa}_\text{mTAL} \), is calculated as the total Na⁺ molar flow (summed over all mTALs) at the inlet (x = L), minus that at the outlet (x = 0).

**Active O₂ Consumption**

The volumetric rate of active O₂ consumption in mTAL epithelia (\( \gamma^\text{active}_\text{mTAL-O}_2 \)) is given by

\[
\gamma^\text{active}_\text{mTAL-O}_2 = \frac{2\pi R^\text{mTAL} \Psi^\text{active}_\text{mTAL}, \text{Na} \theta(P^\text{mTAL}_\text{O}_2)}{18A^\text{epi} \text{mTAL}}
\]  

(9)

where \( R^\text{mTAL} \) and \( A^\text{epi} \text{mTAL} \) denote the mTAL radius and epithelial cross-sectional area, respectively, \( \theta(P^\text{mTAL}_\text{O}_2) \) is the proportion of the active transport rate that is supported by aerobic respiration, and 18 is the number of Na⁺ moles actively reabsorbed per mole of O₂ consumed under maximal efficiency. We assume that (14)

\[
\theta(P^\text{mTAL}_\text{O}_2) = \begin{cases} 
1 & P^\text{mTAL}_\text{O}_2 \geq P_c \\
\frac{P^\text{mTAL}_\text{O}_2}{P_c} & P^\text{mTAL}_\text{O}_2 < P_c
\end{cases}
\]  

(10)

The total consumption rate of O₂ in the OM is determined by integrating \( \gamma^\text{active}_\text{mTAL-O}_2 \) along the full length of short and long mTALs (that is, we neglect basal O₂ consumption). The supply of O₂ to the OM is taken as the total molar flow of O₂ (in free form or bound to Hb) in LDV and SDV at the corticomedullary junction.

**Effects of Hypoxia on NO and O₂⁻**

Oxygen is a substrate for NO synthesis. The volumetric rate of NO generation in compartment \( i \) (\( G^\text{NO}_i \)) is represented by an O₂⁻-dependent Michaelis-Menten relationship

\[
V^\text{active}_\text{mTAL} = \left[ \frac{V^\text{max,Na}_\text{mTAL}}{K^\text{Na}_\text{mTAL} + C^\text{mTAL}_\text{NO}} \right] \cdot f(P^\text{mTAL}_\text{O}_2) \cdot g(C^\text{mTAL}_\text{NO}) \cdot h(C^\text{mTAL}_{O_2^{-}})
\]  

(5)

where \( V^\text{max,Na}_\text{mTAL} \) (in mol Na⁺m⁻²s⁻¹) is the maximal rate of Na⁺ transport, \( K^\text{Na}_\text{mTAL} \) is the Michaelis-Menten constant (140 nM), and the functions \( f(P^\text{mTAL}_\text{O}_2) \), \( g(C^\text{mTAL}_\text{NO}) \), and \( h(C^\text{mTAL}_{O_2^{-}}) \), respectively, represent the effects of O₂, NO, and O₂⁻ on the mTAL reabsorption rate. \( P^\text{mTAL}_\text{O}_2 \) denotes the partial pressure of O₂ in mTALs. The constant \( V^\text{max,Na} \) is estimated as 57.0 mmol/cm²s⁻¹ in the inner stripe, and 23.1 in the outer stripe (14). To simplify the notation, we omit the dependence of the variables on spatial position (x) in Eq. 5, as well as in the equations below.

We assume that when \( P^\text{mTAL}_\text{O}_2 \) falls below a critical value \( P_c \) (taken as 5 mmHg) (5), anaerobic metabolism partly takes

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**Fig. 2. Flow and pressure profiles in long DVR (LDV) and in the short DVR (SDV) that reach the outer-inner medullary junction, for scenario A. Results are similar for both scenarios. x: Position along the corticomedullary axis; x/L = 0 at the corticomedullary junction, and x/L = 1 at the outer-inner medullary junction. A: vessel radius. The radius is a function of luminal NO concentration (Eq. 1). B: blood flow rate. Blood flow variations along the corticomedullary axis are determined based upon the rate of water reabsorption. C: luminal pressure, calculated using the Poiseuille equation (Eq. 2). The pressure gradient is steeper in SDV than in LDV, because it is inversely proportional to the 4th power of the vessel radius.**

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**NaCl Transport Rate**

The axial osmolarity gradient in the OM is generated and maintained by active salt reabsorption in mTALs, which is driven by basolateral Na⁺-K⁺-ATPase pumps. As in our previous model, the rate of active Na⁺ transport along mTALs (\( \Psi^\text{active}_\text{mTAL,Na} \)) is expressed as

\[
\Psi^\text{active}_\text{mTAL,Na} = \frac{V^\text{max,Na}_\text{mTAL}}{K^\text{Na}_\text{mTAL} + C^\text{mTAL}_\text{NO}} \cdot f(P^\text{mTAL}_\text{O}_2) \cdot g(C^\text{mTAL}_\text{NO}) \cdot h(C^\text{mTAL}_{O_2^{-}})
\]  

(5)
that low PO₂ increases O₂ boundary conditions (such as inlet O₂ concentrations) are the same in both scenarios.

Parameter Values

Aside from the effects of NO on DVR radius and blood flow, the model described herein is identical to the one we recently published (14). Tables summarizing parameter values and the description of our numerical method can be found in the latter study.

MODEL RESULTS

This study focuses on the interactions between O₂, NO, and O₂ and the ways in which they affect tubular and vascular function in the OM. We first describe baseline concentration profiles and then simulate variations in production rates or reaction rates to probe the mechanisms by which blood flow and O₂ supply can be modulated by NO to match the energetic requirements of the OM. For each set of simulations, we compare results for two cases: the “vasoactive case,” which accounts for the NO-dependence of DVR radius, and the “fixed R case,” which neglects that dependence.

Base Case

The baseline mTAL Na⁺ concentration profiles, and interstitial fluid O₂, NO, and O₂ concentration profiles are shown in Figs. 3 (scenario A) and 4 (scenario B) for the vasoactive case. Because model parameters were chosen so that baseline DVR inflow rates are analogous in the vasoactive case and the fixed R case, the profiles obtained in these two cases are similar. Both cases predict that the segregation of long DVR and long AVR within vascular bundles in the inner stripe gives rise to significant concentration differences between R1 (the center of the vascular bundles) and R2–R4 (the peripheral regions). The high metabolic requirements of mTALs in the interbundle regions substantially deplete O₂ therein. NO concentrations are predicted to be higher in R1 than in R2 and R3, due to the poor availability of its substrate (i.e., O₂) in the latter regions; the sharp rise in interstitial C_NO in R4 in the inner stripe is caused...
by tubule migration (14). When the O$_2^-$ generation rate ($G_{O_2^-}$) is assumed to decrease with decreasing P$_O_2$ (scenario A), interstitial C$_{O_2^-}$ variations reflect P$_O_2$ variations (see Fig. 3, B and D); when $G_{O_2^-}$ is taken to be independent of P$_O_2$ (scenario B), interstitial C$_{O_2^-}$ varies with the fractional area occupied by vasa recta and descending limbs (i.e., the main suppliers of O$_2^-$) within each region (see Fig. 4D).

Since O$_2^-$ is the predominant NO scavenger in the interbundle region, the fact that C$_{O_2^-}$ is higher in scenario B than in scenario A means that conversely, C$_{NO}$ is lower in R2–R4 than in R1 (compare Figs. 3C and 4C). Hence, NO inhibits NaCl reabsorption to a lesser extent in scenario B, and the concentrating ability of the OM, which is evaluated as the osmolality of the collecting duct fluid at the junction between the outer and inner medulla (denoted osm$_{CD}$), is higher in that scenario than in scenario A (867 vs. 789 mosmol/kgH$_2$O, respectively; see Table 1). Even though mTALs actively reabsorb more NaCl in scenario B, the O$_2$ consumption rate is slightly lower than (43 vs. 44 pmol/s per vascular bundle), because P$_O_2$ decreases more in the inner stripe, so that a greater fraction of the mTAL energetic requirements is supported by anaerobic metabolism. The predicted O$_2$ consumption-to-supply ratio is 0.74 in scenario B and 0.75 in scenario A, in good agreement with the experimental estimate of 0.79 in the rat OM (3). As shown in Figs. 5 (scenario A) and 6 (scenario B), results for the vasoactive case and fixed R case are similar under baseline conditions.

**Indirect Effects of O$_2^-$ on MBF**

The mechanisms by which O$_2^-$ modulates MBF remain to be elucidated (15). Could O$_2^-$-induced vasoconstriction be mediated by NO, at least in part, given that O$_2^-$ reduces NO levels via scavenging, thereby reducing the vasodilatory effects of NO? To probe this issue, we conducted simulations in which we eliminated the direct interactions between NO and O$_2^-$ by

**Table 1. Vascular and tubular function in the rat outer medulla**

<table>
<thead>
<tr>
<th>Scenario A</th>
<th>SDV inflow, n/min</th>
<th>O$_2$ consumption-to-supply ratio</th>
<th>osm$_{CD}$ mosmol/kgH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td>8.0 (8.0)</td>
<td>0.75 (0.76)</td>
<td>789 (787)</td>
</tr>
<tr>
<td>No NO-O$_2^-$ rxn</td>
<td>8.0 (8.0)</td>
<td>0.75 (0.75)</td>
<td>770 (771)</td>
</tr>
<tr>
<td>$G_{NO}$ = 0</td>
<td>7.5 (8.0)</td>
<td>0.82 (0.80)</td>
<td>873 (854)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 2</td>
<td>8.1 (8.0)</td>
<td>0.74 (0.73)</td>
<td>725 (730)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 2</td>
<td>7.8 (8.0)</td>
<td>0.80 (0.78)</td>
<td>856 (845)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 2</td>
<td>8.1 (8.0)</td>
<td>0.77 (0.75)</td>
<td>783 (788)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 5</td>
<td>8.6 (8.0)</td>
<td>0.47 (0.66)</td>
<td>535 (694)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scenario B</th>
<th>SDV inflow, n/min</th>
<th>O$_2$ consumption-to-supply ratio</th>
<th>osm$_{CD}$ mosmol/kgH$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base case</td>
<td>8.0 (8.0)</td>
<td>0.74 (0.74)</td>
<td>867 (867)</td>
</tr>
<tr>
<td>No NO-O$_2^-$ rxn</td>
<td>8.4 (8.0)</td>
<td>0.59 (0.65)</td>
<td>666 (701)</td>
</tr>
<tr>
<td>$G_{NO}$ = 0</td>
<td>7.6 (8.0)</td>
<td>0.78 (0.78)</td>
<td>967 (936)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 2</td>
<td>8.1 (8.0)</td>
<td>0.70 (0.71)</td>
<td>800 (805)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 2</td>
<td>7.6 (8.0)</td>
<td>0.79 (0.79)</td>
<td>1,096 (1,037)</td>
</tr>
<tr>
<td>$G_{NO}$ $\times$ 5</td>
<td>8.5 (8.0)</td>
<td>0.59 (0.71)</td>
<td>755 (893)</td>
</tr>
</tbody>
</table>

$G_{NO}$ and $G_{NO}$: rates of NO and O$_2^-$ synthesis by tubular epithelia; osm$_{CD}$: osmolality of collecting duct fluid at the outer-inner medullary junction. The short descending vasa recta (SDV) inflow is averaged over all SDV. Numbers in parenthesis correspond to the “fixed R case,” which neglects DVR vasoactivity.
setting their reaction rate to zero. We first consider the vasoactive case.

In scenario A, inhibiting the reaction raises CNO (and CO2/H11002) by a few nanomolar in the interbundle region, not enough to raise DVR inflow (Table 1). Since NO and O2/H11002 exert counterbalancing effects on the mTAL transport rate, mNa\textsubscript{mTAL} and PO2 vary only slightly. Thus, in the absence of the NO-O2/H11002 reaction, osmCD\textsubscript{L} and the O2 consumption-to-supply ratio vary by <3% (Table 1).

In scenario B, the consequences of inhibiting the NO-O2\textsuperscript{−} reaction are more pronounced: because CO2/H11002 is two- to threefold higher in this scenario, O2/H11002 scavenging has a greater impact on interbundle NO levels (14). Thus, when the reaction is abolished, CNO increases more (Fig. 7), and the average SDV inflow rises from 8.0 to 8.4 nl/min (LDV inflow varies by 0.04 nl/min). The inhibitory effects of NO on mTAL transport then predominate, TNa\textsubscript{mTAL} and O2 consumption both decrease (Fig. 6), and osmCD\textsubscript{L} is reduced by 23%. Altogether, the O2 consumption-to-supply ratio in scenario B is found to decrease significantly less (from 0.74 to 0.65) when the NO-O2\textsuperscript{−} reaction rate is set to zero. Indeed, the fixed R case does not consider the following positive feedback mechanism: as CNO increases, DVR dilate and carry more O2 into the medulla, which in turn raises the generation rate of NO (G\textsubscript{NO}) and therefore CNO, and so on. This cycle will stop at some point, however, because the extent to which DVR can dilate, and that to which G\textsubscript{NO} can increase, are both intrinsically limited (see the saturable expressions in Eqs. 1 and 11). Moreover, other vasoconstrictor agents, not represented in the present model, are likely to be released to limit such NO-dependent vasodilation. It is also possible that as PO2 increases, hypoxia-induced stimulation of NO release diminishes, thus putting another break on this positive feedback mechanism.

Finally, it should be noted that inhibiting the NO-O2\textsuperscript{−} reaction reduces osmCD\textsubscript{L} less in the fixed R case than in the vasoactive case in scenario B (Table 1): since CNO does not rise as much, mTAL transport is less inhibited. Together, these results suggest that NO-induced vasodilation may significantly affect the O2 balance and the concentrating capacity of the
OM, and that O$_2$-induced DVR vasoconstriction could be at least partly mediated by NO.

**Impact of Epithelial NO Generation**

To assess the contribution of tubular NO generation to the maintenance of medullary oxygenation, we first simulated an isolated twofold increase in the rate of NO synthesis by epithelia ($G_{NO}^{ep}$). Under these conditions, calculated CNO values are 10–20 nM higher in the interbundle region (see Fig. 8 for scenario A). NO-mediated inhibition of active Na$^+$ transport is then enhanced, and osm$_{CP}^L$, T$_{Na}^{mTAL}$, and O$_2$ consumption are predicted to decrease by 4–8% (Figs. 5 and 6). The impact on O$_2$ supply is small, and the O$_2$ consumption-to-supply ratio decreases in proportion to consumption (Table 1).

Conversely, in the absence of NO generation by epithelia, CNO is predicted to decrease by 10–30 nM in R2–R4 (vasoactive case) relative to baseline conditions (Fig. 8). The resulting vasoconstriction of SDV reduces O$_2$ supply to the interbundle region by ~5% (Figs. 5 and 6). In parallel, the rate of active Na$^+$ transport (which is less inhibited by NO) rises by 10%, and so does osm$_{CP}^L$ (Table 1). Overall O$_2$ consumption is predicted to increase only slightly (by ~2%) for the following reason. The increase in T$_{Na}^{mTAL}$ requires more energy, but because O$_2$ supply to the interbundle region decreases, in the vicinity of mTAL P$_O2$ falls even further below the critical pressure along most of the inner stripe (see Fig. 8A), so that a smaller fraction of active Na$^+$ transport is supported by aerobic respiration. In other words, O$_2$ consumption does not increase in proportion to T$_{Na}^{mTAL}$ because a greater fraction of mTAL energy requirements is provided by anaerobic metabolism. Overall, the O$_2$ consumption-to-supply ratio increases to 0.82 (scenario A) and 0.78 (scenario B).

In the fixed R case, the O$_2$ supply to the interbundle region is constant. Thus, in the absence of NO generation by epithelia, P$_O2$ does not fall as significantly in the tissue surrounding mTALs (see Fig. 8, A and C), and O$_2$ consumption increases in proportion to the rate of active Na$^+$ transport, that is, more than in the vasoactive case (Figs. 5 and 6). As a result, the increase in the O$_2$ consumption-to-supply ratio is comparable in both cases (Table 1). Taken together, these results suggest that NO tubulovascular cross talk has a significant impact on OM O$_2$ balance and concentrating capacity.

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**Fig. 6.** Predicted rates of O$_2$ supply, O$_2$ consumption, and mTAL NaCl reabsorption, in pmol·s$^{-1}$·vascular bundle$^{-1}$, for scenario B. Results are shown for different cases: baseline conditions (basal), in the absence of reaction between NO and O$_2$ (no rxn), when $G_{NO}^{ep}$ is 0, 2, or 5 times its baseline value (NO × 0, 2, or 5) , and/or when $G_{O2}^{ep}$ is twice its baseline value (O$_2$ × 2). Black bars, vasoactive case; grey bars, fixed R case.
ANG II-Mediated Increases in Epithelial Generation

We then examined the impact of tubular NO synthesis specifically in response to ANG II. ANG II stimulates the production by mTAL epithelia of not only O$_2^{-}$ but also NO (24, 25). The ANG II-induced increase in NO synthesis is thought to help abrogate tissue hypoxia (31). We simulated first an isolated increase in the tubular production of O$_2^{-}$, followed by an increase in that of both O$_2^{-}$ and NO. Based on experimental measurements of O$_2^{-}$ and NO concentrations in the presence of ANG II (24, 25, 39), epithelial $G_{O2^{-}}$ ($G_{O2^{-}}^{ep})$ was multiplied by a factor of 2, and epithelial $G_{NO}$ ($G_{NO}^{ep})$ by a factor of 2 or 5. Note that we did not consider the NO-independent mechanisms by which ANG II may induce DVR vasoconstriction in these simulations.

As expected, a twofold increase in $G_{O2^{-}}$ raises CO$_2$ in the vicinity of mTALs, thereby stimulating active Na$^+$ transport, and increasing both oxygen consumption (Fig. 5 and 6) and the concentrating capacity of the medulla (Table 1). The rate of NO scavenging by O$_2$ increases concomitantly (more so in scenario B), leading to vasoconstriction of SDV in the

Fig. 7. Impact of the NO-O$_2^{-}$ reaction on concentration profiles (scenario B, vasoactive case). Results are shown for the base case (basal) and assuming that the NO-O$_2^{-}$ reaction rate is zero (no rxn). A: luminal CNa in LAL and SAL. B: interstitial PO$_2$ in regions R2 and R4. C: interstitial CNO in R2 and R4. D: interstitial CO$_2$ in R2 and R4. In the interbundle region, inhibition of the NO-O$_2^{-}$ reaction has a larger impact on CNO than on CO$_2$. The subsequent increase in O$_2$ supply is accompanied by a significant decrease in the rate of NaCl reabsorption across LAL and SAL, and in the rate of O$_2$ consumption.

Fig. 8. Impact of NO synthesis by tubular epithelia on PO$_2$ and CNO in region R3, where most mTALs are located. Results are shown for scenario A. The rate of NO synthesis by tubular epithelia is either equal to its baseline value (basal), multiplied by 2 ($G/2$), or 0 ($G/0$). A and B: PO$_2$ and CNO in the R3 interstitium, accounting for vasoactivity of DVR. C and D: PO$_2$ and CNO in the R3 interstitium, assuming no vasoactivity of DVR. Results suggest that NO tubulovascular cross talk has a significant impact on O$_2$ balance in the outer medulla.
interbundle region and a reduction in local O$_2$ delivery. The O$_2$ consumption-to-supply ratio is thus predicted to increase by 5 percentage points in both scenarios.

A concomitant increase in $G_{\text{NO}}$, however, counteracts the stimulating effects of O$_2$, on NaCl transport and O$_2$ consumption in the OM. A twofold increase in $G_{\text{NO}}$ offsets the $T_{\text{Na}}^{\text{TOTAL}}$ increase, either fully (scenario A) or partially (scenario B) (Figs. 5 and 6). It also raises C$_{\text{NO}}$ sufficiently to essentially abolish SDV constriction and restore O$_2$ supply to the interbundle region. Thus, when $G_{\text{O}_2}^{\text{ep}}$ and $G_{\text{NO}}^{\text{ep}}$ are both doubled, the O$_2$ consumption-to-supply ratio is predicted to remain close to its baseline value (Table 1).

If $G_{\text{NO}}^{\text{ep}}$ is increased five times relative to its baseline level (while $G_{\text{O}_2}^{\text{ep}}$ is doubled), O$_2$ supply to the interbundle region is further augmented, while active Na$^+$ transport and O$_2$ consumption both decrease significantly below baseline levels (Figs. 5 and 6). Under these conditions, the O$_2$ consumption-to-supply ratio is calculated as 0.47 in scenario A and 0.59 in scenario B. The corresponding predictions, displayed in Fig. 9 (case 3), conflict with experimental findings, however; in vivo, acute suppressor doses of ANG II do not significantly alter MBF and medullary Po$_2$ (39). These results thus suggest that ANG II induces comparable increases in $G_{\text{O}_2}^{\text{ep}}$ and $G_{\text{NO}}^{\text{ep}}$.

As expected, the two- or fivefold $G_{\text{NO}}^{\text{ep}}$ increase is predicted to have a lesser impact in the fixed R case (Fig. 9). That is, in the absence of tubulovascular cross talk, $T_{\text{Na}}^{\text{TOTAL}}$, osmCD and the O$_2$ consumption-to-supply ratio are much less reduced (Table 1). Taken together, our results suggest that ANG II-mediated NO synthesis acts not only to counterbalance the effects of ANG II on vasoconstriction, as observed experimentally (39, 36), but also to reduce active Na$^+$ transport and thereby preserve O$_2$ availability in the OM.

**DISCUSSION**

The effects of NO and O$_2$ on vascular and tubular function in the OM are difficult to untangle: not only do NO and O$_2$ exert opposite actions on blood flow and sodium transport while reducing each other’s bioavailability (15) but they also interact with O$_2$ in ways that are not fully understood. In this study, we used a mathematical model to gain more insight into the reciprocal interactions between O$_2$, NO, and O$_2$.

The most significant limitations of our model stems from the paucity of experimental data. As previously discussed (14), absolute O$_2$ concentrations in the renal medulla have not yet been reported; the quantitative effects of NO and O$_2$ on mTAL reabsorption rates are also uncertain. Similarly, the data we extrapolated to predict the effects of C$_{\text{NO}}$ on DVR diameter and blood flow (Eq. 1) are somewhat limited. In addition, the contribution of anaerobic metabolism to the energy requirements of the OM, which likely varies among species, has never been precisely determined. Further experimental studies are needed to fill those gaps, and to elucidate the effects of downstream products such as ONOO$^-$ (peroxynitrite) and H$_2$O$_2$ (hydrogen peroxide) on MBF (23, 26), which our current model does not consider. Finally, conflicting experimental results regarding the effects of low Po$_2$ on medullary O$_2$ synthesis (9, 22), as well as the uncertainty surrounding the mechanisms by which hypoxia raises medullary NO levels, also require additional investigation.

To circumvent some of these limitations, we considered two scenarios in parallel throughout the study: scenarios A and B assume that O$_2$ synthesis respectively decreases and increases with decreasing Po$_2$. Since medullary Po$_2$ is low, C$_{\text{O}_2}^{\text{ep}}$ is significantly higher in scenario B. This distinction proved useful when we examined the indirect effects of O$_2$ on MBF. Our results suggest that O$_2$-induced vasoconstriction may be partly mediated by NO, provided that O$_2$ levels are sufficiently high. When the scavenging effects of O$_2$ on NO were eliminated, SDV inflow was predicted to remain constant in scenario A, and to increase by 5% in scenario B (from 8.0 to 8.4 nl/min).

Our model predicts differential regulation of vasoactivity in short and long DVR. The region-based approach allows us to take into account the specific architecture of the rat OM, with

**Fig. 9.** Impact of epithelial production of O$_2$ ($G_{\text{O}_2}^{\text{ep}}$) and NO ($G_{\text{NO}}^{\text{ep}}$) on Po$_2$ and C$_{\text{NO}}$ in region R3. Results are shown for scenario A. Basal denotes baseline profiles. In case 1, $G_{\text{O}_2}^{\text{ep}}$ is multiplied by 2 (relative to its baseline value); in case 2, $G_{\text{O}_2}^{\text{ep}}$ and $G_{\text{NO}}^{\text{ep}}$ are both multiplied by 2. In case 3, $G_{\text{O}_2}^{\text{ep}}$ is multiplied by 2, and $G_{\text{NO}}^{\text{ep}}$ by 5. A and B: Po$_2$ and C$_{\text{NO}}$ in the R3 interstitium, accounting for vasoactivity of DVR. C and D: Po$_2$ and C$_{\text{NO}}$ in the R3 interstitium, assuming no vasoactivity of DVR. Results suggest that ANG II-induced stimulation of NO synthesis by mTAL counteracts the effects of oxidative stress.
its sharp distinction between intra- and interbundle regions. The LDV, which are destined to the inner medulla, are positioned at the center of the vascular bundles, whereas the SDV peel off from the bundle periphery to supply blood flow to the OM capillary plexus. In all the simulations performed for this study, LDV inflow remained approximately constant (it was always comprised between 7.9 and 8.1 nl/min) even when SDV inflow varied significantly. That is, the segregation of LDV within the center of the vascular bundles is predicted to insulate these vessels from changes in tubular function in the OM, thereby preserving O2 delivery to the inner medulla.

We also found that the impact of the interactions between O2, NO, and O2− is enhanced by a positive feedback mechanism, the importance of which remains to be ascertained in vivo. Since O2 is a precursor in the synthesis of NO, an increase in PO2 should raise NO concentrations, thereby augmenting DVR diameter, blood flow, and O2 supply; this should in turn further raise CNO, and so on. This positive feedback loop should nevertheless be mitigated, if not abolished, by the combination of several factors: 1) the limited extent to which DVR can dilate, which this model accounts for; 2) other vasoconstrictor agents such as endothelins, not considered in this study; and 3) the extent to which hypoxia affects NO bioavailability. Heyman et al. (17) observed an increase in medullary NO levels when PO2 was lowered. Hypoxia-induced NO release has been reported in other tissues and is thought to be a mechanism whereby local perfusion is adjusted to match O2 requirements (1). The underlying signaling pathways are still controversial and remain to be fully elucidated (1, 7, 8, 12). The tight coupling between PO2 and CNO illustrated by the model underscores the importance of better understanding the effects of hypoxia on NO bioavailability in the outer medulla.

We used the current model to assess in particular the importance of tubulovascular cross talk in maintaining OM perfusion and O2 availability. Cowley and colleagues (36, 39) showed that ANG II-induced increases in NO production offset the vasoconstrictor effects of ANG II and maintain MBF constant. Our results suggest that even basal production of NO by tubular epithelia acts to preserve blood flow and O2 supply to the interbundle region (Figs. 5 and 6). In the absence of tubular NO synthesis and subsequent NO diffusion to neighboring DVR, O2 supply to the interbundle region is predicted to decrease by 5%, and the O2 consumption-to-supply ratio to grow by 4–7% (Table 1). These differences are not huge, but they are significant in light of the hypoxic conditions that prevail in the OM. The variations in the O2 ratio stem not only from the reduction in O2 supply but also from changes in O2 consumption, as the inhibitory effects of NO on active Na+ transport across mTALs are abrogated; in the absence of tubular NO synthesis, the rate of NaCl reabsorption and the concentrating capacity of the outer medulla are calculated to be ~10% higher (Table 1). In other words, NO acts to preserve O2 availability in the interbundle region by modulating both oxygen supply and demand, not just by matching O2 delivery to the metabolic needs of mTALs. In the presence of ANG II, the combined regulation of O2 consumption and supply by NO is predicted to significantly attenuate the hypoxic effects of ANG II (Fig. 9), as postulated by other investigators (28, 31).

Our investigations also suggest that O2 consumption may not systematically increase in proportion to the rate of active Na+ transport, in the presence of anaerobic metabolism. In those simulations where PO2 in the vicinity of mTALs was below the critical pressure in most of the inner stripe (where the rate of NaCl reabsorption is the highest), the rate of O2 consumption did not rise as fast as TNa−. This prediction is contingent upon our assumption that anaerobic metabolism can be a significant source of ATP in the rat OM. The current model assumes that, when necessary, glycolysis may produce enough energy to sustain half the maximal transport rate (i.e., that at which O2 is not rate limiting; see Eq. 6). Ex vivo, the production of lactate by isolated rat mTAL segments was found to increase markedly following incubation with the oxidative metabolism inhibitor antimycin A (2). Whether, in vivo, anaerobic metabolism is sufficient to sustain an increased rate of NaCl reabsorption even as PO2 decreases remains to be ascertained. At the very least, the supply of glucose to the OM seems more than sufficient to satisfy the corresponding ATP requirements. Assuming a glucose concentration of 10 mM in DVR blood (37), the supply of glucose to the OM is 80 pmol·min−1·DVR−1, or 4.6 nmol·min−1·vascular bundle−1. The basal rate of Na+ transport across mTALs, estimated as 1,200 pmol Na+·min−1·bundle−1 (Figs. 5 and 6), requires 400 pmol ATP·min−1·bundle−1, which in turn necessitates the conversion of 200 pmol glucose-min−1·bundle−1 under anaerobic conditions, which is less than one-tenth of the glucose supply.

In conclusion, this model underlines the importance of O2 availability in regulating the balance between NO and O2 and their effects on vascular and tubular function in the rat OM. Conversely, our results suggest that basal production of NO by tubular epithelia modulates both the supply and demand of O2 in the interbundle region.

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

AUTHOR CONTRIBUTIONS
Author contributions: A.E. and A.T.L. provided conception and design of research; A.E. and A.T.L. analyzed data; A.E. and A.T.L. interpreted results of experiments; A.E. prepared figures; A.E. drafted manuscript; A.E. and A.T.L. edited and revised manuscript; A.E. and A.T.L. approved final version of manuscript; A.T.L. performed experiments.

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