Determinants of basal nitric oxide concentration in the renal medullary microcirculation

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Zhang, Wensheng, Tosapol Pibulsonggram, and Aurélie Edwards. Determinants of basal nitric oxide concentration in the renal medullary microcirculation. Am J Physiol Renal Physiol 287: F1189–F1203, 2004.—In this study, we modeled the production, transport, and consumption of nitric oxide (NO) in the renal medullary microcirculation under basal conditions. To yield agreement with reported NO concentrations of ~60–140 nM in medullary tissues (Zou AP and Cowley AW Jr. Hypertension 29: 194–198, 1997; Am J Physiol Renal Integr Comp Physiol 279: R769–R777, 2000) and 3 nM in plasma (Stamler JS, Jaraiki O, Osborne J, Simon DL, Keaney J, Vita J, Singel D, Valeri CR, and Loscalzo J. Proc Natl Acad Sci USA 89: 7674–7677, 1992), the permeabilities of red blood cells (RBCs), vascular walls, and pericytes to NO are all predicted to lie between 0.01 and 0.1 cm/s, and the NO production rate by vasa recta endothelium is estimated to be on the order of 10⁻¹⁴ μmol·μm⁻²·s⁻¹. Our results suggest that the concentration of NO in RBCs, which is essentially controlled by the kinetics of NO scavenging by hemoglobin, is ~0.01 nM, that is, 10³ times lower than that in plasma, pericytes, and interstitium. Because the basal concentration of NO in pericytes is on the order of 10 nM, it may be too low to activate guanylate cyclase, i.e., to induce vasorelaxation. Our simulations also indicate that basal superoxide concentrations may be too low to affect medullary NO levels but that, under pathological conditions, superoxide may be a very significant scavenger of NO. We also found that although oxygen is a negligible NO scavenger, medullary hypoxia may significantly enhance NO concentration gradients along the corticomedullary axis. Kidney: mathematical model; transport; medullary hypoxia

Nitric oxide (NO) has been identified as a regulator of signal transduction and vascular tone for two decades. In the renal medulla, three isoforms of nitric oxide synthase (NOS) have been found: endothelial (eNOS), neuronal (nNOS), and cytokine inducible (iNOS). Wu et al. (64) observed that the concentration of NO in tissues of the renal medulla and as 31–95 nM in the cortex. NO concentrations of ~60–140 nM in medullary tissues (Zou AP and Cowley AW Jr. Hypertension 29: 194–198, 1997; Am J Physiol Renal Integr Comp Physiol 279: R769–R777, 2000) and 3 nM in plasma (Stamler JS, Jaraiki O, Osborne J, Simon DL, Keaney J, Vita J, Singel D, Valeri CR, and Loscalzo J. Proc Natl Acad Sci USA 89: 7674–7677, 1992), the permeabilities of red blood cells (RBCs), vascular walls, and pericytes to NO are all predicted to lie between 0.01 and 0.1 cm/s, and the NO production rate by vasa recta endothelium is estimated to be on the order of 10⁻¹⁴ μmol·μm⁻²·s⁻¹. Our results suggest that the concentration of NO in RBCs, which is essentially controlled by the kinetics of NO scavenging by hemoglobin, is ~0.01 nM, that is, 10³ times lower than that in plasma, pericytes, and interstitium. Because the basal concentration of NO in pericytes is on the order of 10 nM, it may be too low to affect medullary NO levels but that, under pathological conditions, superoxide may be a very significant scavenger of NO. We also found that although oxygen is a negligible NO scavenger, medullary hypoxia may significantly enhance NO concentration gradients along the corticomedullary axis. Kidney: mathematical model; transport; medullary hypoxia

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leagues (53) simulated the transport of NO in renal afferent arterioles and predicted that the advective transport of locally produced NO is sufficient to yield physiologically significant NO concentrations along the arteriole. Given the size of vasa recta and their countercurrent arrangement, the concentration of NO in the medullary microcirculation is expected to be governed by different transport processes. In this study, we modeled the generation, transport, and consumption of NO in vasa recta to gain a better understanding of the determinants of basal NO concentration in the renal medulla.

GLOSSARY

- $C_i^j$: Concentration of solute $i$ in compartment $j$, $j = R, P, PC, I$
- $D$: Diameter of vasa recta
- $FN$: Fraction of the surface area occupied by fenestrations in vasa recta wall
- $f_{NO}^i$: Fraction of NO generated by vasa recta endothelium that diffuses toward the lumen
- $G_i^j$: Net reactive production rate of component $i$ in compartment $j$, $j = R, P, PC, I$
- $h$: Hematocrit
- $H$: Thickness of pericyte
- $Hb$: Heme
- $HbO_2$: Oxyheme
- $HbNO$: Nitrosyl-heme
- $IN%$: Percentage inhibition of NO synthesis
- $J_i^j$: Flux of solute $i$ through membrane $j$, $j = R, W, PC$
- $L$: Length of renal medulla
- $L_k^i$: Hydraulic conductivity of pathway $k$ in compartment $j$, $j = R, W$
- $met-Hb$: Metheme
- $n$: Hill constant
- $P_i^j$: Permeability of component $i$ across membrane $j$, $j = R, W, PC$
- $Q_i$: Volume flow rate in compartment $j$
- $R_i$: Reaction rate of reaction $i$
- $SNO-Hb$: S-nitrosohemoglobin

Greek Symbols

- $\Gamma$: Red blood cell-to-vessel surface area ratio
- $\Pi$: Oncotic pressure of proteins
- $\gamma_i$: Activity coefficient of solute $i$
- $\sigma_i$: Reflection coefficient to solute $i$
- $\Psi_i^j$: Generation rate of component $i$ in compartment $j$

Subscripts and Superscripts

- $A$: Ascending vasa recta
- $D$: Descending vasa recta
- $I$: Interstitium
- $IMCD$: Inner medullary collecting duct
- $mTAL$: Medullary thick ascending limb
- $P$: Plasma
- $PC$: Pericyte
- $R$: Red blood cell
- $W$: Vascular wall

MODEL AND PARAMETERS

General Description of Model

Figure 1 represents the countercurrent exchange system of the renal medullary microcirculation. Blood flows down along the DVR from the corticomedullary junction to the papillary tip and loops back to the cortical veins along the ascending vasa recta (AVR). During transit, radial exchanges between vasa recta and the medullary interstitium allow water and solutes deposited from the loops of Henle and the collecting ducts into the interstitium to be carried away by the medullary microcirculation.

Our model, which has been described and applied earlier (13, 65), consists of steady-state conservation equations for water and solutes in red blood cells, vasa recta, and interstitium coupled with expressions for fluxes across vasa recta walls and red blood cell (RBC) membranes through paracellular and transcellular pathways. We only consider those vasa recta that are destined for the inner medulla (IM), i.e., those that lie in the center of the vascular bundles and do not perfuse the capillary plexus in the outer medulla (OM). The numbers of DVR and AVR vary with medullary depth. The reabsorption of water and solutes from nephron loops into the medullary interstitium is accounted for by interstitial generation rates.

In the renal medulla, NO is predominantly produced by vasa recta, IMCDs, and mTALs (64). A portion of NO generated within vasa recta walls is assumed to diffuse into the vessel lumen, where it reacts with scavengers such as oxygen, superoxide, and the hemoglobin contained within red blood cells. The remainder diffuses abluminally toward the interstitium. In outer medullary DVR, NO has to diffuse across pericytes before it arrives in the interstitium; within the pericytes, a fraction of NO reacts with superoxide and oxygen, as well as with guanylate cyclase (GC) to induce vasodilation, and the rest reaches the medullary interstitium. NO generated by IMCDs and mTALs is also assumed to diffuse into the interstitium. These additional sources of interstitial NO, combined with the rapid degradation of NO in blood, result in a significant concentration gradient across the vascular wall that drives NO into the lumen. Our assumptions regarding the synthesis, transport, and consumption of NO are given below. The gen-

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![Diagram](http://ajprenal.org)
eral mathematical model of transport within the medullary microcirculation has been described previously (65), and our modifications to include NO are described in the APPENDIX.

**NO Synthesis in the Renal Medulla**

NO is produced from a group of enzymes called NOS that catalyze a five-electron oxidation of the guanidine nitrogen of L-arginine by NADPH and O$_2$ (20). Three isoforms of the NOS enzyme, that is, nNOS (or NOS I), iNOS (or NOS II), and eNOS (or NOS III), have been identified throughout different structures of the renal medulla. All three enzymes can coexist in a given region and contribute to the overall production of NO within that region (64). There are numerous regulatory pathways at both the transcriptional and posttranslational levels that make pinpointing key biological factors of NO production extremely difficult.

Wu et al. (64) estimated the production of NO in the renal medulla by measuring the production of L-citrulline from microdissected segments in the rat kidney. The NO generation rates from IMCDs, vasa recta, and mTALs were reported as 11.5, 3.2, and 0.5 fmol·mm$^{-1}$·h$^{-1}$, respectively, based on 20-mm-long segments. Those values are equivalent to $3.4 \times 10^{-17}$, $2.2 \times 10^{-17}$, and $0.22 \times 10^{-17}$ μmol·μm$^{-2}$·s$^{-1}$, respectively, based on the average surface area of IMCDs (0.094 mm$^2$/mm length), DVR and AVR (0.041 mm$^2$/mm length), and mTALs (0.063 mm$^2$/mm length), given the diameters and AVR-to-DVR number ratio listed in Table 1. Based on their mathematical model of NO transport, Vaughn et al. (62) calculated the NO production flux from nonspecific vascular endothelial cells as $6.8 \times 10^{-14}$ μmol·μm$^{-2}$·s$^{-1}$. Their estimate is therefore three orders of magnitude greater than that of Wu et al. (64). Because of this discrepancy, the rate of NO generation in vasa recta was a variable parameter in this study.
Nitric Oxide in Vasa Recta

\[ NO + GC \rightarrow GC - NO \]  \hspace{1cm} \text{(reaction 5)}

\[ \text{HbO}_2 \leftrightarrow \text{Hb} + O_2 \]  \hspace{1cm} \text{(reaction 6)}

The corresponding reaction rates can be written as

\[ R_1 = -\frac{d[\text{NO}]}{dr} = k_5[\text{NO}][\text{O}_2] \]  \hspace{1cm} (1)

\[ R_2 = -\frac{d[\text{NO}]}{dr} = k_6[\text{NO}][\text{O}_2] \]  \hspace{1cm} (2)

\[ R_3 = -\frac{d[\text{NO}]}{dr} = k_7[\text{NO}][\text{HbO}_2] \]  \hspace{1cm} (3)

\[ R_4 = -\frac{d[\text{NO}]}{dr} = k_8[\text{NO}][\text{Hb}] - k_{-4}[\text{HbNO}] \]  \hspace{1cm} (4)

\[ R_5 = -\frac{d[\text{NO}]}{dr} = k_9[\text{GC}][\text{NO}] \]  \hspace{1cm} (5)

\[ R_6 = -\frac{d[\text{HbO}_2]}{dr} = k_6[\text{HbO}_2] - k_{-6}[\text{Hb}][\text{O}_2] \]  \hspace{1cm} (6)

Following the approaches of Vaughn et al. (62) and Butler et al. (5), the rate of reaction between NO and GC was assumed to be first order with respect to NO concentration, as the GC concentration was assumed to be constant. The kinetic rate product \( k_5[\text{GC}] \) was taken as 0.01 s\(^{-1}\) and the perycine thickness as 0.5 \( \mu \text{m} \), as described below.

The hemoglobin molecule is composed of four subunit proteins, two \( \alpha \) and two \( \beta \), each containing an iron-based heme group that can bind one \( \text{O}_2 \) or NO molecule. The mechanisms by which NO and \( \text{O}_2 \) bind to hemoglobin are complex and remain to be fully elucidated. In this study, we made the simplifying assumption that each individual heme group can be treated separately and that each reacts with NO independently of the binding state of the other groups. Hereafter, we use the term Hb to denote one heme protein group, as opposed to the entire hemoglobin molecule, unless otherwise specified. Therefore, the overall concentration of heme proteins is four times that of hemoglobin. NO oxidizes the Fe(II) in oxyheme (\( \text{HbO}_2 \)) to form metheme (met-Hb; reaction 3), whereas deoxyheme (Hb) and NO associate to form ligand-bound nitrosyl-heme (HbNO; reaction 4) in a reversible manner (4). Cassoly and Gibson (6) found no cooperativity in the binding of NO to deoxyhemoglobin; that is, the intrinsic NO addition rate constants do not vary with NO saturation; because the biological concentration of NO is at least 1,000-fold lower than that of heme, most hemoglobin molecules do not carry any NO, and those that do will carry no more than one (49). Therefore, \( k_{-4} \) was taken as 10\(^{-7}\)/s, that is, the value of the kinetic constant for the disassociation of the T-state Hb\(_4\)(NO) (52).

Following the approach of Baxley and Hellums (2), the kinetic constant \( k_{-6} \) was taken as 3.5 \( \times \) 10\(^{6}\) M/s. We derived \( k_6 \) from the Hill equation

\[ k_6 = k_{-6}[\text{O}_2]^{-n}C_{50}^n \]  \hspace{1cm} (7)

where \( C_{50} \) is the half-saturation and \( n \) is the Hill equation parameter, taken as 4.12 \( \times \) 10\(^{-5}\) M and 2.6, respectively (2, 65).

### NO Transport Resistance

As NO enters plasma, there are four diffusive steps leading to its reaction with hemoglobin in RBC: 1) diffusion through the RBC-free region created by intravascular flow, toward the bulk solution; 2) diffusion from the bulk solution to the surface of the RBC (extracellular layer); 3) diffusion across the RBC membrane; and 4) diffusion and reaction in the cytosol.

Subczynski et al. (56) measured the permeability of artificial lipid membranes to NO. Their results, on the order of 77–93 cm/s, suggest that the RBC membrane itself (i.e., step 3) is not rate limiting. Vaughn et al. (60) examined the extracellular resistance to NO transport by comparing the reaction rate of NO with hemoglobin either within RBCs (that is, in a process involving steps 2–4 above) or in a continuous, cell-free solution (that is, with only extracellular diffusion). If the hematocrit was at least 7.5%, the apparent RBC-to-cell-free hemoglobin solution kinetic constant ratio was found to be 0.001, independently of the hematocrit. This suggests that the main barriers to NO transport are the RBC membrane and cytosol and that the resistance to extracellular diffusion is comparatively negligible. The permeability of RBC barriers (membrane and cytosol) to NO (\( P_{SO} \)) was therefore taken as 0.04 cm/s, as estimated by Vaughn et al. (61). In the absence of experimental measurements of the permeability of vasa recta walls to NO (\( P_{SO}^\text{W} \)), we used the same estimate as that to oxygen, 0.04 cm/s (23, 65).

We assumed that the wall permeability implicitly accounts for the resistance of the boundary layer in the RBC-free zone adjacent to the wall (i.e., step 1 above), which has been shown to reduce NO consumption (34). In the absence of data, we also assumed that the permeability of pericytes (membrane and cytosol) to NO is the same as that of RBCs, 0.04 cm/s.

### Initial Values of NO and Scavenger Concentration

Zou and Cowley (67, 68) measured tissue NO concentrations as 57–139 nM in the renal medulla and 31–95 nM in the cortex. In addition, the concentration of NO in plasma was reported as 3 nM by Stamler et al. (54). Given the high concentration of heme protein groups (on the order of 20 mM) which scavenge NO almost instantaneously, NO concentration is expected to be much lower in RBC than in plasma. As a first trial, we assumed that the initial values (i.e., at the corticomedullary junction in DVR) of \( C_{SO}^n \) and \( C_{NO}^n \) are 1 nM and 0, respectively.

The initial concentration of overall heme species was taken as 20 mM (65). However, the distribution of Hb proteins at the corticomedullary junction is unknown. Because both the reaction between deoxyheme and NO (reaction 4) and the association between \( \text{O}_2 \) and Hb (reaction 6) are reversible, we assumed that they are in equilibrium at the corticomedullary junction and calculated the initial value of Hb, HbNO, and \( \text{HbO}_2 \) concentrations based on this hypothesis, after having specified \( C_{SO}^n \). If initial \( C_{SO}^n \) is assumed to be zero, the initial concentration of HbNO is also zero.

\( \text{O}_2 \)-producing enzymes, such as NADH/NADPH oxidase, mitochondrial enzymes, and xanthine oxidase (XO), have been...
pericytes are disposed at intervals of 14–20 μm (57), we assumed that pericytes surround either OMDVR only, as suggested by Pallone and Silldorff (47), or both OMDVR and IMDVR, because Takahashi-Iwanaga (57) observed pericytes in the upper portion of IMDVR.

The resulting medullary NO concentration profiles in all compartments are shown in Fig. 2A for the baseline case (pericytes in OM only) and in Fig. 2B, assuming that pericytes are present throughout the medulla.

In the baseline case, \( C_{NO} \) is calculated to be about twice as high in interstitium and pericytes as in plasma and \( \sim 10^3 \) greater in plasma than in RBCs. Because we assumed constant generation rates, \( C_{NO} \) varies within a narrow range in each compartment. The predicted concentrations of NO in interstitium and pericytes (\( \sim 20 \) nM) and in plasma (\( \sim 8 \) nM) are comparable to the measurements of Zou and Cowley (67, 68) in medullary tissue (60–140 nM) and those (54) in plasma (3 nM). The abrupt variations at the OM-IM junction observed in Fig. 2A stem from the sudden disappearance of pericytes at that location. As seen in Fig. 2B, when pericytes are taken to surround both OMDVR and IMDVR, NO concentration profiles are smoother, but overall NO levels remain similar to those in the baseline case.

The large plasma-to-RBC \( C_{NO} \) gradient is due to the very rapid scavenging of NO by hemoglobin. RBCs act as a sink for NO; as shown below, the barriers formed by the RBC membrane and cytosol and the vascular wall prevent the rapid depletion of NO in plasma and interstitium. Because the RBC concentration of deoxygenated Hb is higher in AVR than in DVR given the release of oxygen for metabolic consumption, and because NO reacts with deoxygenated Hb, NO concentrations are lower in AVR than in DVR both in plasma and RBCs.

Given the small concentration variations within each compartment, in the remainder of this study NO concentration is reported as the average value for each compartment. When the differences in \( C_{NO} \) between OM and IM are significant, concentrations in the OM and IM are reported separately.

We then performed a parameter sensitivity analysis by examining the effect of increasing and decreasing each of the parameters by 50% on NO concentration in each compartment. Results, shown in Table 2, indicate that the determinant parameters are the NO generation rates, the fraction of NO generated in vasa recta endothelium that diffuses toward the medullary tissues, which range between 60 and 140 nM (67, 68), and in plasma, reported as 3 nM (54).

Baseline values of the parameters are listed in Table 1. As explained below, NO generation rate in vasa recta was taken as \( 10^{-14} \mu\text{mol} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1} \). NO induces vasorelaxation by acting on the pericytes that surround DVR. The outer surface of DVR is only partially covered with pericytes whose thickness is 2.5 μm in the cross section that includes their nucleus and 0.7 μm in that which contains their tentacles (Dr. Tom L. Pallone, personal communication). To account as well for the fact that pericytes are disposed at intervals of 14–20 μm (57), we assumed in the baseline case that they are 0.5 μm thick but form a continuous layer. We examined two hypotheses, namely, that pericytes surround either OMDVR only, as suggested by Pallone and Silldorff (47), or both OMDVR and IMDVR, because Takahashi-Iwanaga (57) observed pericytes in the upper portion of IMDVR.

RESULTS

As described above, several parameters related to NO transport remain uncertain, including the NO production rate by the endothelium of vasa recta and epithelium of mTALs and IMCDs, the permeability of vasa recta walls and cell membranes to NO, \( O_2^2 \) concentrations in the medulla, and the dimensions of pericytes. In this study, we examined the effects of all these factors on basal NO concentrations in the renal microvasculature, and we also sought to find parameter values that yielded agreement between our predictions and measurements of NO concentration both in medullary tissues, which range between 60 and 140 nM (67, 68), and in plasma, reported as 3 nM (54).

Fig. 2. Basal nitric oxide (NO) concentrations as a function of position along the corticomedullary axis (\( x \)); \( L \) is the total length of the medulla. The generation rate of NO in vasa recta is taken as \( 10^{-14} \mu\text{mol} \cdot \mu\text{m}^{-2} \cdot \text{s}^{-1} \). \( A \): pericytes cover DVR in outer medulla (OM) only. \( B \): pericytes cover DVR in both OM and inner medulla (IM).
interstitium. Because Wu et al. (64), calculations fail to converge.†Results were obtained by varying superoxide concentration in RBC, plasma, interstitium, and pericytes separately.

increasing $f_{NO}$, the initial hematocrit (h₀), and the permeabilities of vascular walls, pericytes, and RBCs in decreasing order of importance. We therefore studied the effects of those parameters first.

**NO Production Rates by Endothelium of Vasa Recta and Epithelium of mTALs and IMCDs**

Wu et al. (64) measured the NO generation rate by vasa recta ($\Psi_{NO}^{v}$), mTALs ($\Psi_{NO}^{mTAL}$), and IMCDs ($\Psi_{NO}^{IMCD}$) as 3.2, 0.5, and 11.5 fmol·mm⁻¹·h⁻¹, which are equivalent to $2.2 \times 10^{-17}$, $0.22 \times 10^{-17}$, and $3.4 \times 10^{-17}$ µmol·µm⁻²·s⁻¹, respectively. The value for $\Psi_{NO}^{v}$ is $2.2 \times 10^{-17}$ µmol·µm⁻²·s⁻¹, which is approximately 3,000 times lower than that estimated by Vaughn et al. (62) for nonspecific vascular endothelial cells, $6.8 \times 10^{-14}$ µmol·µm⁻²·s⁻¹. Using the generation rates obtained by Wu et al. (64), the predicted $C_{NO}$ in plasma and interstitium is on the order of 0.01 nM, which is several orders of magnitude lower than experimental reports. We therefore increased each parameter ($\Psi_{NO}^{v}$, $\Psi_{mTAL}^{NO}$, and $\Psi_{IMCD}^{NO}$) in turn while keeping the other two fixed at the values given above. The effects of varying $\Psi_{NO}^{v}$, $\Psi_{mTAL}^{NO}$, and $\Psi_{IMCD}^{NO}$ on average medullary NO concentrations are shown in Fig. 3A, B, and C, respectively.

Figure 3A suggests that medullary NO concentrations in all compartments increase almost linearly with increasing $\Psi_{NO}^{v}$. As $\Psi_{NO}^{v}$ is raised from $1 \times 10^{-10}$ to $1 \times 10^{-13}$ µmol·µm⁻²·s⁻¹, $C_{NO}$ increases from $10^{-4}$ to $10^{-2}$ nM in RBCs, from $10^{-3}$ to 80 nM in plasma, and from 0.01 to 200 nM in pericytes and interstitium. Because $\Psi_{NO}^{v}$ is much greater than both $\Psi_{NO}^{mTAL}$ and $\Psi_{NO}^{IMCD}$ in these simulations, vasa recta endothelium is the main source of NO; the latter diffuses from the vascular wall into the pericytes and then into the interstitium, so that C_NO is slightly higher in pericytes than in OM interstitium. Because $\Psi_{NO}^{v}$ is much greater than both $\Psi_{NO}^{mTAL}$ and $\Psi_{NO}^{IMCD}$, the latter diffuses from the vascular wall into the pericytes and then into the interstitium, so that C_NO is slightly higher in pericytes than in OM interstitium. Our results suggest that with $\Psi_{NO}^{mTAL}$ and $\Psi_{NO}^{IMCD}$ fixed at the values measured by Wu et al. (64), $\Psi_{NO}^{v}$ should be on the order $10^{-14}$ µmol·µm⁻²·s⁻¹, that is close to the estimate of Vaughn et al. (62), to match experimental measurements of NO concentration in medullary tissue and plasma.

Because mTALs and IMCDs are located in OM and IM, respectively, increasing $\Psi_{NO}^{IMCD}$ raises $C_{NO}$ in all four compartments in the outer medulla only, as shown in Fig. 3B, whereas increasing $\Psi_{NO}^{mTAL}$ raises $C_{NO}$ in the inner medulla only (Fig. 3C). The concentration of NO in pericytes does not vary with $\Psi_{NO}^{mTAL}$ because the latter are taken to surround OMDVR only (baseline hypothesis).

As described below, even if we optimize the values of all the other uncertain parameters to raise medullary $C_{NO}$, predictions based on the data of Wu et al. (64) cannot match the experimental measurements of both Zou and Cowley (67, 68) and Stampler et al. (54). Agreement between calculated and reported values of $C_{NO}$ is possible only if we increase $\Psi_{NO}^{mTAL}$, and/or $\Psi_{IMCD}^{NO}$. However, increasing the three generation rates simultaneously would introduce many more uncertainties. We therefore chose to raise only $\Psi_{NO}^{v}$ and chose a value of $1 \times 10^{-14}$ µmol·µm⁻²·s⁻¹ as our baseline case, while keeping $\Psi_{mTAL}^{NO}$ and $\Psi_{IMCD}^{NO}$ fixed at $0.22 \times 10^{-13}$ and $3.4 \times 10^{-13}$ µmol·µm⁻²·s⁻¹, respectively, as reported by Wu et al. (64).

**Effects of Fraction of NO Generated by Vasa Recta Wall That Diffuses Toward the Vascular Lumen**

In our baseline case, we assumed that the fraction of NO generated by vasa recta endothelium that diffuses luminally (i.e., $f_{NO}$) is equal to that which diffuses abuminally. Sensitivity analysis indicates that $f_{NO}$ affects $C_{NO}$ significantly (Table 2). Shown in Fig. 4 is the average $C_{NO}$ in plasma, interstitium and pericytes, as $f_{NO}$ is varied between 0 and 100%. Plasma NO concentrations remain approximately constant as equilibrium between plasma and RBCs is maintained, whereas $C_{NO}$ in pericytes and interstitium decreases linearly, by a factor $\sim 3$ as $f_{NO}$ increases from 0 to 100%. In the limiting case where $f_{NO} = 100\%$, that is, when all NO generated by the vascular endothelium is released into the lumen, $C_{NO}$ is higher in DVR plasma than in interstitium. Because experimental measurements (67, 68) suggest that the concentration of NO is significantly greater in interstitium than in blood, lower values of $f_{NO}$ appear more reasonable.

**Effects of Initial Hematocrit**

Variations in hematocrit affect not only the overall amount of hemoglobin but also the surface area at the interface be-

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**Table 2. Parameter sensitivity analysis**

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<th>Parameters</th>
<th>CNO in RBC</th>
<th>CNO in Plasma</th>
<th>CNO in Interstitium</th>
<th>CNO in Pericytes</th>
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</tbody>
</table>

*Variations (expressed as percentage of baseline value) in NO concentration ($C_{NO}$) in RBC, plasma, interstitium, and pericytes obtained by increasing (+) and decreasing (−) each parameter in the left column by 50%, while all other parameters are kept constant and equal to their baseline values. †Generation rate; $f_{NO}$, fraction of NO generated by vasa recta endothelium that diffuses toward the lumen. *With initial hematocrit taken as 12.5% (i.e., a 50% decrease), calculations fail to converge. †Results were obtained by varying superoxide concentration in RBC, plasma, interstitium, and pericytes separately.
tween plasma and RBCs (65). An increase in the initial hematocrit at the corticomedullary junction increases the volume of RBCs, hence the cell-to-wall surface area ratio, thereby lowering NO concentration differences between plasma and RBCs. Because the latter act as a sink for NO, the larger surface area for exchange leads to a significant reduction in plasma NO concentration, hence in $C_{\text{NO}}$ in interstitium and pericytes. As shown in Fig. 5, a twofold increase in the initial hematocrit (i.e., from 25 to 50%) lowers medullary $C_{\text{NO}}$ by ~50% in RBC and plasma and ~25% in interstitium and pericytes.

Effect of Permeability to NO

The RBC (membrane and cytosol) permeability to NO ($P_{\text{NO}}^{\text{R}}$) was taken as 0.04 cm/s (61). In the absence of reported measurements, the permeability of the pericytic cells to NO ($P_{\text{NO}}^{\text{PC}}$) was also taken as 0.04 cm/s, and we assumed that the permeability of the vasa recta wall to NO ($P_{\text{NO}}^{\text{W}}$) is equal to that to oxygen, 0.04 cm/s (23, 65). Given these uncertainties, we varied each permeability individually from 0.005 to 0.5 cm/s.
(as explained below) while keeping the other two constant. Shown in Fig. 6, A and B, are the average NO concentrations in the medulla as a function of $P_{NO}^{R}$ and $P_{NO}^{P}$, respectively.

As $P_{NO}^{R}$ increases from $5 \times 10^{-3}$ to 0.5 cm/s, $C_{NO}$ decreases significantly in plasma, pericyte, and interstitium but remains unchanged in RBCs (Fig. 6A). Indeed, in this permeability range, the amount of NO scavenged in plasma is negligible relative to that diffusing into RBCs (as evinced by the fraction of NO consumed by superoxide and hemoglobin, respectively, given in Table 3); hence, if the vasa recta production rate of NO remains unchanged, the flux of NO from plasma to RBCs stays constant to satisfy mass conservation. The flux is proportional to the product of $P_{NO}^{R}$ and $\Delta C_{NO} = C_{NO}^p - C_{NO}^i$; because NO concentration in RBC is $\sim 1,000$-fold lower than in plasma, $\Delta C_{NO} \approx C_{NO}^i$, so that the product of the permeability and the plasma NO concentration remains constant. We were not able to reduce $P_{NO}^{R}$ below $5 \times 10^{-3}$ cm/s. Indeed, very low $P_{NO}^{R}$ values result in higher NO concentrations throughout the outer medulla; if $C_{NO}^i$ is too large in the OM, oxygen is depleted before the papillary tip due to the balance between NO, Hb, and HbO$_2$, and simulations fail to converge.

Similarly, increasing $P_{NO}^{P}$ reduces NO concentrations in pericytes and interstitium but does not affect significantly $C_{NO}^i$ in plasma and RBCs for the most part (Fig. 6B). As $P_{NO}^{P}$ becomes $>0.1$ cm/s, NO concentrations in plasma begin to increase due to the very significant influx of NO from pericytes and/or interstitium; the concentration differences between AVR plasma and interstitium then become very small [i.e., $C_{NO}^i(\text{AVR}) \approx C_{NO}^i(\text{PC})$, whereas that between pericytes and interstitium remains large because $P_{NO}^{PC}$ is fixed at 0.04 cm/s. As a consequence, $C_{NO}^i(\text{AVR})$ increases more rapidly than and surpasses $C_{NO}^i(\text{DVR})$. As $P_{NO}^{P} > 0.5$ cm/s, simulations fail to converge for the same reasons as above.

We then varied $P_{PC}$ while keeping $P_{NO}^{R}$ and $P_{NO}^{P}$ fixed at 0.04 cm/s. As $P_{PC}$ increases from 0.005 to 0.5 cm/s, $C_{NO}^i$ remains approximately constant in RBCs, plasma, and interstitium and decreases by $<20\%$ in pericytes. This decrease is not very significant because the pericytes occupy a comparatively small volume (i.e., 13% of that of OMDVR lumen and 1% of that of the overall vascular lumen). These results indicate that the permeability of the RBC to NO is an important determinant of NO concentration in plasma, pericytes, and interstitium and that the permeability of vascular wall to NO significantly affects $C_{NO}^i$ in interstitium and pericytes. To raise interstitial NO concentrations, we would have to lower our baseline permeability values; however, assuming that $\Psi_{NO}$ were as low as $10^{-17}$ $\mu$mol $\mu$m$^{-2}$s$^{-1}$ as reported by Wu et al. (64), NO concentration would remain close to 1 nM in the interstitium and pericytes even if $P_{NO}^{W}$, $P_{NO}^{R}$, and $P_{NO}^{PC}$ were as small as $5 \times 10^{-3}$ cm/s, a value that seems too low given that NO and O$_2$ are similar in size and that the permeability of RBCs and the vascular wall to oxygen is predicted to be on the order of $10^{-2}$ cm/s (23, 65).

### $O_2$ Concentration

We assumed in this study that the concentration of superoxide remains fixed in each compartment throughout the medulla; in the baseline case, it was taken as 0.1 nM (see above). Among the species considered here, superoxide scavenges NO at a much higher rate than do oxygen and GC. In RBCs,
however, the relative amount of NO scavenged by O$_2^-$ is negligible. Hence, decreasing the concentration of superoxide in RBCs to zero or increasing it to 1 nM has a negligible effect on the RBC concentration of NO (results not shown).

As shown in Table 3, reducing O$_2$ concentrations in all compartments to zero raises medullary NO levels by <1% in all compartments. Conversely, a 10-fold increase in C$_{O_2}$ in all compartments reduces NO concentrations by ~6% in plasma and interstitium and by ~3% in pericites, whereas the fraction of NO scavenged by O$_2^-$ increases from ~0.6% in the baseline case to ~6%. Increasing O$_2^-$ concentration in each compartment separately shows that only plasma and interstitial C$_{O_2}^-$ have a measurable effect on medullary NO levels (Table 3). A 100-fold increase in all compartments increases the fraction of NO scavenged by O$_2^-$ by 60% and decreases C$_{NO}$ by ~90%.

To the best of our knowledge, measurements of O$_2^-$ concentration in the renal medulla have not been reported. Our results suggest that agreement between our model predictions and NO levels reported in the literature can only be obtained with NO production rates on the order of 10$^{-14}$ μmol·μm$^{-2}$·s$^{-1}$, because decreasing the concentration of O$_2^-$ has little effect on that of NO. With lower generation flux values, no agreement is possible independently of O$_2^-$ concentration.

**Initial NO Concentration**

The initial value (i.e., at the corticomedullary junction in DVR) of plasma C$_{NO}$ was chosen as 1 nM based on reported measurements of NO concentration in plasma (54). Our simulations indicate that this initial value has a negligible effect on average NO concentrations in the medulla, as a 10-fold increase or decrease in this parameter does not affect C$_{NO}$ in interstitium, plasma, and RBCs, independently of the NO generation rate in vasa recta (results not shown).

As described above, the initial value of C$_{NO}^R$ was chosen as 0. Over the range of $\Psi_{NO}^R$ values that we examined, our simulations did not converge if we increased the initial C$_{NO}^R$ to 0.1 nM. Indeed, because we assumed in our baseline case that both HbO$_2$ and nitrosyl-heme are at equilibrium with NO and O$_2$ at the corticomedullary junction in DVR, increasing the initial C$_{NO}$ in RBCs simultaneously raises C$_{HbNO}$ and reduces C$_{Hb}$. This enhances the dissociation of HbO$_2$ and thus raises the concentration of free O$_2$ at the junction; O$_2$ is then shunted from DVR to AVR in the OM and is therefore depleted before blood reaches the papillary tip. Instead of the equilibrium hypothesis, we then assumed that the initial concentration of HbNO is 0; under these conditions, our simulations suggest that raising the initial value of C$_{NO}^R$ from 0 to 1 nM does not affect the average C$_{NO}$ in the four compartments. Given that the rate constant for the binding of NO to Hb is 10 times larger than that for the dissociation of HbNO, the equilibrium hypothesis (namely, that NO and HbNO are at equilibrium at the corticomedullary junction) is more likely to be true than the alternate assumption (namely, that the concentration of HbNO is 0 at the junction).

With $\Psi_{NO}^R$ equal to its baseline value of 10$^{-14}$ μmol·μm$^{-2}$·s$^{-1}$, increasing the initial C$_{NO}^R$ from 0 to 0.01 nM only raises the average C$_{NO}$ from 0.010 to 0.013 nM and does not affect NO concentration in other compartments. However, if $\Psi_{NO}^R$ is on the order of 10$^{-17}$ μmol·μm$^{-2}$·s$^{-1}$, the same increase in the initial C$_{NO}^R$ raises the average NO concentration in RBCs from 2 × 10$^{-5}$ to 10$^{-3}$ nM. Indeed, because we assumed that at the corticomedullary junction, HbNO is in equilibrium with NO and Hb, and HbO$_2$ is in equilibrium with O$_2$ and Hb, the combination of these two reactions yields the following balance at the junction: HbO$_2$ + NO $\Leftrightarrow$ HbNO + O$_2$ (so that variations in the initial C$_{HbNO}$ result in similar changes in the initial C$_{HbNO}$). Although this equilibrium is not strictly maintained beyond the corticomedullary junction, deviations from it appear to be small, and our model suggests that NO is never completely scavenged by HbO$_2$ (reaction 4) even though this scavenging reaction is not reversible. When the NO vasa recta production rate is high, NO influx from plasma is high, so that reaction of Hb and NO goes forward. With lower values of $\Psi_{NO}^R$, however, the NO influx is so low so that the reaction is reversed, that is, HbNO actually dissociates to release NO; as described above, the latter is not completely scavenged by HbO$_2$. In that sense, the reversible association of Hb and O$_2$ plays a significant role in maintaining NO concentrations in RBCs.

Because variations in initial C$_{NO}^R$ and C$_{NO}^P$ values do not significantly affect plasma and interstitial NO levels, these parameters cannot be optimized to obtain agreement between calculated and measured concentrations if $\Psi_{NO}^P$ is significantly lower than 10$^{-14}$ μmol·μm$^{-2}$·s$^{-1}$.

**Dependence of NO Synthesis on PO$_2$**

Our simulations suggest that the fraction of NO scavenged by O$_2$ is negligible relative to that consumed by hemoglobin and O$_2^-$. As a substrate in the synthesis of both NO and O$_2^-$, O$_2$ nevertheless plays an important role in NO transport. A NO-mediated increase in blood flow increases the supply of O$_2$ and therefore P$_O_2$, which in turn affects medullary C$_{NO}$. As such, there is a strong correlation between O$_2$ and NO concentrations in the renal medulla. In studies of cultured bovine aortic endothelial cells, Whorton et al. (63) measured a concentration-dependent decrease in NO production as P$_O_2$ was reduced and observed that the percentage of inhibition of NO production as a function of P$_O_2$ was a sigmoidal curve, other than a Michaelis-Menten profile, if Ca$^{2+}$ was saturated. A regression of the experimental data obtained by Whorton et al. (63) yields the following expression

$$IN\% = \frac{91 - 10}{1 + \exp[(P_{O_2} - P_{1/2})/5.1]} + 10$$

(8)

where IN% is the percent inhibition of NO production by endothelial cells, and P$_{1/2}$ is defined as P$_O_2$ at which IN% equals 50. Whorton et al. (63) reported P$_{1/2}$ as 38 Torr, based on asymptotic values of 100 and 0, whereas using their actual upper and lower limits (91 and 10, respectively), we estimated P$_{1/2}$ as 34 Torr.

According to the review of Buerk (4), the O$_2$-dependent NO generation rate abides by the conventional Michaelis-Menten equation. The corresponding inhibition ratio can be expressed as

$$IN\% = \frac{P_{1/2}}{P_{1/2} + P_{O_2}} \times 100$$

(9)

The Michaelis constant for NO synthesis by eNOS has been reported as 7.7 (50) and 88 μM (35), corresponding to P$_{1/2}$ = 5 and 56 Torr, respectively, assuming that the solubility of O$_2$ is 1.56 μM/Torr (7). That for NO synthesis by nNOS has been
measured as 23 (50) and 400 μM (1), that is, $P_{1/2} = 15$ and 256 Torr, respectively. Assuming that nNOS represents 30–50% of all NOS isoforms in the renal medulla (37), the upper limit of $P_{1/2}$ should thus be ~150 Torr.

Shown in Fig. 7A are interstitial NO concentration profiles along the corticomedullary axis in our baseline case (case a, no inhibition), using Eq. 8 based on the data of Whorton et al. (63) (case b), and assuming a Michaelis-Menten expression as given by Eq. 9 with $P_{1/2}$ as 34 and 150 Torr (cases c and d), respectively. The interstitial $P_{O2}$ is predicted as 46 Torr at the corticomedullary junction and ~20 Torr at the papillary tip (Fig. 7B). When NO synthesis is correlated with $P_{O2}$, NO concentration decreases significantly along the corticomedullary axis. For equal values of $P_{1/2}$, the decrease in $C_{NO}$ is much steeper if Eq. 8 is used rather than the Michaelis-Menten expression, as shown by a comparison between cases b and c. However, NO concentrations at the papillary tip are similar using Eq. 8 with $P_{1/2} = 34$ Torr and Eq. 9 with the upper limit $P_{1/2} = 150$ Torr (cases b and d). The average NO concentration in interstitium is calculated to be 2.9, 6.6, and 2.2 nM in cases b, c, and d, respectively, vs. 16.9 nM in the baseline case (case a).

The concentration of NO in the study of Whorton et al. (63) was measured indirectly in vitro under saturated conditions for endothelial $Ca^{2+}$ and l-arginine. The relationship between NO generation and $P_{O2}$ in vivo is likely to be much more complex than that described by Eqs. 8 and 9, with many other factors involved. For example, the dependence of ROS concentration on $P_{O2}$ must also be considered. Our model cannot currently accurately predict the reciprocal effects of $C_{NO}$ and $P_{O2}$ on each other. However, these results suggest that medullary hypoxia is likely to significantly affect NO concentrations in the renal medulla.

**DISCUSSION**

The production of NO in the renal medulla protects it from hypoxic injury not only by maintaining perfusion (and thus $O_2$ supply) but also by inhibiting salt reabsorption in the thick ascending limb, thereby reducing $O_2$ consumption (47). Infusion of l-NG-monomethylarginine (l-NMMA), an inhibitor of NO formation, decreases medullary $P_{O2}$ from 23 to 12 Torr (3)

In the medullary microvasculature, NO acts by regulating the vascular tone and the solute permeability of vasa recta (39), both of which are important factors in controlling arterial pressure and blood flow. In this study, we modeled the transport of NO in the renal medullary microcirculation to gain fundamental insight into the determinants of basal NO concentration in vasa recta. The production of NO by vasa recta, IMCDs, and mTALs, the transport of NO across the cellular membrane and cytosol as well as the vascular wall, and NO-scavenging reactions in plasma, RBCs, pericytes, and interstitium were considered. We examined the effects of changes in hematocrit, NO production rates, and permeability to NO on NO levels in the renal medulla, as well as the inhibition effect of low $P_{O2}$ on NO production.

Our investigation was limited to parametric studies because there is very little experimental data related to NO in the renal medulla. We did not find in the literature estimates of several important variables, such as medullary $O_2$ levels. $O_2$ concentrations have been reported in arterial blood and intracellularly (10, 28) but may be significantly different in vasa recta given medullary hypoxia. Zou et al. (69) found that $O_2$ is primarily produced in the OM but did not report $O_2$ concentrations. Other uncertain parameters include the permeability of vasa recta, erythrocytes, and pericytes to NO, the volume, thickness, and distribution of pericytes, as well as kinetic constant values at 37°C. As summarized by Buerk (4), the kinetics of NO-scavenging reactions have been observed mostly at 20°C. In simulating the consumption of NO within erythrocytes, Tsoukias and Popel (59) extrapolated kinetic constant values to 37°C assuming a temperature coefficient of 1.4/10°C (that is, a 68% increase from 20 to 37°C) based on data for CO binding to deoxyhemoglobin reported by Cassoly and Gibson (6). Given the uncertainty of this approach, we opted to use the kinetic constant values at 20°C consistently and examined the effects of variations in these parameters on NO transport. Our simulations indicate that varying the kinetic rate constants of the reactions between NO and hemoglobin affects the relative amounts of NO scavenged by deoxyheme and oxyheme but has

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*Fig. 7. Effect of $P_{O2}$ inhibition of NO synthesis on NO concentration ($C_{NO}$). Under $O_2$ saturation conditions, the NO generation rate ($V_{NO}$) is taken as $10^{-14}$ μmol·μm$^{-2}$·s$^{-1}$. A: NO concentration as a function of medullary depth. B: $P_{O2}$ as a function of medullary depth. a: Baseline case: no inhibition, $V_{NO}$ remains constant along the corticomedullary axis. b: Inhibition ratio for $V_{NO}$ is given by Eq. 8, with $P_{O2}$ at which percent inhibition of NO production by endothelial cells (IN%) equals 50 ($P_{1/2} = 34$ Torr. c: Inhibition ratio for $V_{NO}$ is given by Eq. 9, with $P_{1/2} = 34$ Torr. d: Inhibition ratio for $V_{NO}$ is given by Eq. 9, with $P_{1/2} = 150$ Torr.*
a negligible effect on NO concentrations in plasma, pericytes, and interstitium.

In addition, changes in medullary NO concentrations have not been measured simultaneously with changes in DVR diameter, blood flow, or PO2. We were therefore not able to correlate these variables and to simulate the effect of NO on variations in capillary size and O2 supply. We also did not model the effects of NO on the tubular system, including sodium reabsorption and oxygen consumption by mTALs.

Because the half-life of NO in vivo is a few milliseconds, NO should only be able to exert its effects within a few micrometers of its site of generation (17). Evidence to the contrary suggests that NO may be stored under forms that preclude its inactivation, and the conversion of NO into S-nitrosothiols (RSNOs) has been postulated to play an important role in its preservation (17, 25). RSNOs are thioesters of nitrite with the generic structure R-S-N=O, and S-nitrosoalbumin is thought to account for most of the circulating RSNOs (17). Although estimates of RSNO concentration in plasma vary widely, they are generally believed to remain under 0.15 μmol/l in control subjects (17). The mechanisms by which RSNOs are formed in vivo and by which they may release NO are unclear (25). Pawloski and colleagues (48, 49) have suggested that S-nitrosohemoglobin (SNO-Hb) is formed in RBCs by transferring NO from the nitrosyl group of the hemoglobin molecule to the highly conserved ß-chain cysteine 93 residue (cys-Hb), and that under anaerobic conditions, the nitroso moiety is transferred to the chloride-bicarbonate anion-exchanger 1 (AE1) protein, which carries it from the erythrocyte to vascular muscle cells to elicit vasodilation. Given that the suggested mechanisms of SNO-Hb formation remain speculative (17, 18, 29), and in the absence of experimental kinetics data on RSNO formation, we did not account for the effects of RSNOs in this model.

Our results are also limited by our compartmental approach, in that we did not take into account the radial NO concentration gradients within each compartment, which are likely to be significant because NO is a highly active free radical. Considering both radial and axial variations along the corticomedullary axis, however, is beyond the scope of this study.

Observations suggest that pericytes are disposed at intervals of 14–20 μm along DVR (57) and that their thickness varies from ~2.5 μm across their nucleus to 0.7 μm across their tentacles (Dr. Tom L. Pallone, personal communication). Our baseline assumption that pericytes form a continuous, 0.5-μm-thick layer surrounding OMDVR is therefore oversimplified. However, changes in their thickness, distribution, and O2 concentration are all predicted to have a negligible effect on medullary NO levels; even when the permeability of pericytes to NO increases from 0.005 to 0.5 cm/s, pericyte CNO is reduced by only 20%, because pericytes occupy <1% of the vascular lumen volume. Although NO regulates vascular tone at the level of the pericytes, our simulations suggest that these cells do not significantly affect NO concentrations in the renal medulla.

Our results indicate that basal NO concentration in pericytes is ~20 nM. The concentration of NO above which the vasodilator is able to activate GC has been reported to lie between 23 and 120 nM, as reviewed by Tsoukas et al. (58), and a value as high as 250 nM was also reported by Stone and Marletta (55). Our results thus suggest that basal NO levels are too low to activate GC and induce vasodilation. Decreasing the permeability of RBCs or vascular walls to NO, or increasing the endothelial generation rate of NO would raise CNO in pericytes, but the latter concentration would remain well below 250 nM. These results are not surprising; under basal conditions, NO is not expected to induce vasodilation.

We assumed in this study that NO is generated by both DVR and AVR endothelium. Because AVR have a highly fenestrated endothelium, it is possible that they lack the cellular machinery needed to regulate NOS; the activity of NOS has been found to be affected by the presence of caveolae (31), which may be absent in AVR. To investigate this hypothesis, we performed simulations in which the NO generation rate in AVR was eliminated. Results (not shown) indicate that the average CNO in plasma, RBC, and interstitium would then decrease by ~60% relative to the baseline case, whereas that in pericytes would decrease by ~40%. NO concentration profiles along the corticomedullary axis would remain similar, which suggests that the other trends described in this study would still hold.

Our model predicts that the basal concentration of NO in the RBCs of the renal medulla is ~0.01 nM, due to rapid scavenging by hemoglobin. Under physiological conditions, it is the continuous generation of NO in vasa recta walls as well as the low permeability of the RBC membrane and cytosol to NO that allow basal plasma NO concentrations to be maintained at much higher levels. The precise nature of the significant resistance to NO transport across the RBC remains unclear (60). Our parametric studies indicate that the permeability of RBCs and vasa recta walls to NO should both be on the order of 0.01 cm/s to yield agreement with experimental measurements of NO concentration in medullary tissue. The three-orders-of-magnitude difference in NO concentration between erythrocytes and plasma suggests that the two compartments should be carefully distinguished; the term “biological NO concentration in blood” used by many investigators must be employed with caution.

Both experimental and theoretical limitations did not allow us to capture the multiple ways in which NO and O2 interact, such as the fact that O2 is a precursor in the synthesis of not only NO but also of O2−, an important NO scavenger, or the effects of NO-mediated changes in blood flow rate on O2 supply. However, a simple simulation suggested that O2 could play an important role as a substrate for NO production. We found that if the production flux of NO decreases with decreasing PO2 as observed in vitro by Whorton et al. (63), the average NO concentration in plasma and pericytes is about six times lower than in the baseline case (i.e., assuming no inhibition). We did not consider the possible effect of low PO2 on O2− concentration. Indeed, we found that under basal conditions, the scavenging of NO by O2− is small compared with that by hemoglobin (Table 3). However, our simulations also suggest that if O2− concentration is increased 100-fold, CNO decreases by a factor of 10 in all compartments. Under pathological conditions, the production of O2− by vascular endothelium and smooth muscle has been shown to increase >1,000-fold (11, 22), and our results suggest that O2− may then play a significant role in inactivating NO. Under these conditions, the effect of PO2 on O2− concentrations should be taken into account.

The production rate of NO was measured specifically in the renal medulla by Wu et al. (64), who incubated microdissected
renal segments with l-arginine and cofactors and measured NOS activity. However, using their data, predicted medullary NO concentrations were at least one order of magnitude smaller than experimental measurements in plasma made by Stamler et al. (54) and in renal medullary tissues made by Zou and Cowley (67, 68). The release of NO is subject to short- and long-term regulation, and it remains uncertain whether the estimates of Wu et al. (64) adequately reflect basal conditions. To obtain agreement between experimental measurements of NO concentration (54, 67, 68) and our calculations, it was necessary to assume that the vasa recta production of NO is \( \sim 1,000 \) times larger than that measured by Wu et al. (64), i.e., close to the estimates that Vaughn et al. (62) also obtained by mathematical simulation.

It is possible that the estimates of medullary NO concentration reported by Zou and Cowley (67, 68) were too high. However, it has been argued that the microdialysis technique that these investigators used underestimates NO production (21). Moreover, the measurements of Zou and Cowley (67, 68), 50–140 nM, are significantly lower than estimates of NO concentration in other parts of the microcirculation; micromolar concentrations have been reported in rat aorta (36).

It should also be noted that the study of Wu et al. (64) suggests that IMCD is the segment with the greatest NOS enzymatic activity. Dickhout et al. (12) observed that NO produced by tubular epithelial cells can be transported to and enzymatic activity. Dickhout et al. (12) observed that NO produced by tubular epithelial cells can be transported to and play a role in DVR pericytes. Our simulations suggest that IMCD is the segment with the greatest NOS concentration in other parts of the microcirculation; micromolar concentrations have been reported in rat aorta (36).

To obtain agreement between experimental measurements of NO concentration (54, 67, 68) and our calculations, it was necessary to assume that the vasa recta production of NO is \( \sim 1,000 \) times larger than that measured by Wu et al. (64), i.e., close to the estimates that Vaughn et al. (62) also obtained by mathematical simulation.

In summary, our model predicts that basal NO concentrations in renal medullary RBCs are predominantly controlled by the kinetics of NO scavenging by hemoglobin. NO concentration was found to be \( \sim 0.01 \text{ nM} \) in RBCs, on the order of nanomolar in plasma, and \( \sim 20 \text{ nM} \) in pericytes and interstitium. Our results also suggest that a low RBC membrane permeability to NO and a significant NO generation rate in vasa recta are essential for preserving large plasma-to-RBC NO concentration gradients and maintaining NO concentration within pericytes at levels just below that needed to activate GC and regulate vascular tone. Experimental determinations of medullary O\(_2\) concentration, the permeability of RBCs and vasa recta walls to NO, and NO production rates in vasa recta and tubules, as well as more specific measurements of NO concentration in pericytes and interstitium, are needed to significantly extend our mathematical model of NO transport in the medullary microcirculation.

**APPENDIX**

**Mathematical Model**

Our model of the renal medullary microcirculation consists of a series of conservation equations in plasma, RBCs, interstitium, and pericytes, together with flux equations. Because NO reacts with one of the four heme protein groups on the hemoglobin molecule, each heme group, such as deoxyheme (Hb), oxyheme (HbO2), nitrosyl-heme (HbNO), and metheme (met-Hb), is treated separately. In this study, we consider NO, O\(_2\), and urea as the only solutes that cross the RBC membrane. Solutes in plasma include sodium chloride, urea, plasma proteins, O\(_2\), and NO, all of which traverse vasa recta walls through a paracellular pathway shared with water. We also account for two additional pathways across RBC membranes and DVR walls: transcellular aquaporin-1 (AQP1) water channels and UTB urea transporters.

**Conservation Equations**

If \( x \) is the axial coordinate along the corticomedullary axis, conservation of volume in plasma and RBCs can be expressed as

\[
\frac{dQ^p}{dx} = \pm (J^w_\text{pr} - J^w_\text{R}) \pi D + \left( \frac{Q^p}{N} \right) \frac{dN}{dx} \quad (A1)
\]

\[
\frac{dQ^R}{dx} = \pm J^w_\text{R} \pi D + \left( \frac{Q^R}{N} \right) \frac{dN}{dx} \quad (A2)
\]

where \( Q^p \) and \( Q^R \) are the plasma and RBC flow rates, respectively, and \( J^w_\text{pr} \) and \( J^w_\text{R} \) are the volume fluxes (per unit membrane area) across vasa recta walls (positive if directed from vasa recta to interstitium) and RBC membranes (positive if directed from RBC to plasma), respectively. The parameter \( \Gamma \) represents the cell-to-vessel surface area ratio, \( N \) denotes the number of vasa recta and \( D \) their diameter, and \( + \) and \( - \) apply to AVR and DVR, respectively. The second term on the righthand side of Eqs. A1 and A2 accounts for the fact that at various depths in the medulla, DVR break up to form a capillaryplexus, from which AVR are formed and ascend. Hence, part of the flow is directly shunted from DVR to AVR at various levels.

Conservation of solutes in plasma can be expressed as

\[
\frac{d(Q^p C_i^p)}{dx} = \pm (J^w_\text{pr} C_i^p - J^w_\text{R} C_i^p) \pi D + \left( \frac{Q^p C_i^p}{N} \right) \frac{dN}{dx} \quad (A3)
\]

where \( J^w_\text{pr} \) and \( J^w_\text{R} \) are the molar fluxes of solutes through vasa recta walls and RBC membrane, respectively, and \( G_i^p \) is the net generation rate of solute \( i \) in plasma. As above, \( - \) applies to DVR. Because sodium chloride and plasma proteins are absent from RBCs, \( J^w_\text{R} \) and \( J^w_\text{pr} \) are 0. O\(_2\) is consumed by reacting with NO (reaction 1); NO is generated by NOS from vasa recta walls and consumed by O\(_2\) and O\(_2\) in plasma (reactions 1 and 2). Thus we have

\[
G_{O_2} = - \frac{R}{4} (1-h) \frac{N_{\pi}}{4} D^2 \quad (A4)
\]
where \( R_1 \) and \( R_2 \) are obtained by substituting NO and O2 concentrations in Eqs. 1 and 2, \( h \) is the hematocrit, \( \Psi_{\text{NO}}^p \) is the generation rate of NO per unit surface area of vasa recta wall, and \( f_{\text{NO}} \) is the fraction of generated NO which diffuses toward plasma. The parameter \( F \) denotes the fenestration ratio, that is, the fraction of vasa recta wall occupied by fenestrations. Based on data summarized by Michel (42), it is taken as 0 for DVR and 0.3 in AVR. For all other solutes, \( G_i^p = 0 \) because they are neither generated nor consumed by chemical reaction. The solute conservation equation in RBCs may be expressed as

\[
\frac{d(Q^p C_i^p)}{dx} = \pm J_{\text{PR}}^i \Gamma \pi D \pm G_i^p + \left( \frac{Q^p C_i^p}{N} \right) \frac{dN}{dx} \tag{A6}
\]

where for urea, the RBC flow rate should be multiplied by the fractional volume of distribution of urea within RBCs, taken to be 0.86 (8). For all heme groups and other nonurea solutes, \( J_{\text{PR}}^i = 0 \) because they cannot permeate the RBC membrane. In erythrocytes, O2 is consumed by reaction 1 and produced by reaction 6, whereas NO is consumed by reactions 1–4. The net generation rate for heme, oxyheme, nitrosyl-heme, and solutes, \( G_i^p \) is calculated by

\[
G_i^p = \left( R_i - R_1 \right) h \frac{\Gamma \pi D}{4} \tag{A7}
\]

\[
G_{\text{NO}}^p = - \left( R_1 + R_2 + R_3 + R_4 \right) \frac{h \Gamma \pi D}{4} \tag{A8}
\]

The net generation rate for heme, oxyheme, nitrosyl-heme, and metheme can be obtained from reactions 3–5

\[
G_i^p \text{Hb} = \left( R_i - R_4 \right) h \frac{\Gamma \pi D}{4} \tag{A9}
\]

\[
G_i^p \text{Hb02} = - \left( R_1 + R_3 \right) \frac{h \Gamma \pi D}{4} \tag{A10}
\]

\[
G_i^p \text{NO} = R_i h \frac{\Gamma \pi D}{4} \tag{A11}
\]

\[
G_i^p \text{methem} = R_i h \frac{\Gamma \pi D}{4} \tag{A12}
\]

where in Eqs. A7–A12, \( R_1 \)–6 are obtained by substituting RBC concentrations into Eqs. 1–6. If reabsorption from the loops of Henle and collecting ducts is accounted for by interstitial generation rates, conservation of volume, NaCl, urea, proteins, O2, and NO in the interstitium can be written as (13)

\[
\left[ J_{\text{PR}}^i(x) N(x) \pi D \right]_{\text{DVR}} + \left[ J_{\text{PR}}^i(x) N(x) \pi D \right]_{\text{AVR}} + G_i^p = 0 \tag{A13}
\]

For water, sodium, and urea, the interstitial generation term \( G_i^p \) may be expressed as

\[
G_i^p = A_{\text{int}} \Psi_i^p \quad i = v, \text{Na, urea} \tag{A14}
\]

where \( A_{\text{int}} \) is the cross-sectional area of the medullary interstitium, and \( \Psi_i^p \) the generation rate of water, sodium, or urea per unit area of interstitium, accounts for reabsorption from nephrons. The generation rate for proteins is 0, assuming that proteins are exclusively exchanged between vasa recta and interstitium. Calculations related to \( \Psi_i^p \) were described in detail by Edwards et al. (13).

O2 in the medullary interstitium is consumed for metabolic purposes, mainly for active sodium reabsorption, and by reacting with NO. Interstitial NO is generated by NOS synthesis from vasa recta, IMCDs, and mTALs and reacts with O2 and \( O_2^5 \), as expressed by

\[
\frac{G_{\text{NO}}}{(1 - F_{\text{NO}}) \Psi_{\text{NO}}^p N \pi D} \times \left( R_1 + R_2 \right) \frac{h \Gamma \pi D}{4} \tag{A5}
\]

\[
\frac{G_{\text{NO}}}{(1 - F_{\text{NO}}) \Psi_{\text{NO}}^p N \pi D} \times \left( R_1 + R_2 \right) \frac{h \Gamma \pi D}{4} \tag{A15}
\]

\[
G_{\text{NO}} = \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p (1 - F_{\text{NO}}) N \pi D \right]_{\text{DVR}} + \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p \right] \times \left( 1 - F_{\text{NO}} \right) N \pi D \frac{f_{\text{NO}}}{N_{\text{MC}} + N_{\text{MTAL}}} \tag{A16}
\]

\[
G_{\text{NO}} = \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p (1 - F_{\text{NO}}) N \pi D \right]_{\text{DVR}} + \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p \right] \times \left( 1 - F_{\text{NO}} \right) N \pi D \frac{f_{\text{NO}}}{N_{\text{MC}} + N_{\text{MTAL}}} \tag{A17}
\]

\[
G_{\text{NO}} = \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p (1 - F_{\text{NO}}) N \pi D \right]_{\text{DVR}} + \left[ (1 - F_{\text{NO}}) \Psi_{\text{NO}}^p \right] \times \left( 1 - F_{\text{NO}} \right) N \pi D \frac{f_{\text{NO}}}{N_{\text{MC}} + N_{\text{MTAL}}} \tag{A18}
\]

where the subscript PC denotes the pericyte compartment. \( f_{\text{NO}}^p \) is the NO flux from pericytes to interstitium, and \( f_{\text{NO}}^p \) represents the NO flux from plasma to pericytes (Eq. A17) or from plasma to interstitium (Eq. A18); their expressions are given below in Eqs. A26, A28, and A29. The consumption rate of NO in pericytes (\( G_{\text{NO}}^p \)) can be written as a function of the reaction rates of NO with O2, O2, and GC

\[
G_{\text{NO}}^p = - (R_1 + R_4 + R_4) \frac{h \Gamma \pi D}{4} \tag{A19}
\]

where \( H \) is the thickness of pericytes, assumed to be 0.5 mm in the baseline case. Because for each solute, the flux terms in Eqs. A13, A17, and A18 are implicit functions of interstitial and/or pericyte concentrations (or of the interstitial hydraulic pressure in the case of water), solving those equations yields interstitial and pericyte solute concentrations.

**Flux Equations**

The volume flux across the capillary wall, \( J_{\text{PR}}^i \), is the sum of volume fluxes across paracellular pathways and AQP1 water channels, expressed as

\[
J_{\text{PR}}^i = L_{\text{w}}^i \left( \Delta P - \sigma_{\text{pr}} \Delta \Pi_{\text{pr}} \right) + L_{\text{a}}^w \left( \Delta P - \Delta \Pi_{\text{pr}} - RT \sum_{i = \text{sodium area}} \gamma_i (C^i - C_{\text{eq}}^i) \right) \tag{A20}
\]

where \( L_{\text{w}}^i \) and \( L_{\text{a}}^w \) represent the hydraulic conductivities of the paracellular pathway and AQP1, respectively. The superscript \( D \) signifies that AQP1 is expressed in DVR only, so that the second term on the right-hand side is taken to be 0 in AVR. \( \Delta P \) is the transmural hydraulic pressure difference, \( \Delta \Pi_{\text{pr}} \) is the transmural oncotic pressure difference due to plasma proteins, and \( \sigma_{\text{pr}} \) is the reflection coefficient of the paracellular pathway to proteins. The interstitial concentration and the activity coefficient of solute \( i \) are denoted by \( C_i \) and \( \gamma_i \), respectively.

If \( L_{\text{R}} \) represents the overall hydraulic conductivity of the RBC membrane, the water flux across the RBC membrane may be expressed as

\[
J_{\text{PR}}^w = L_{\text{R}} \left( \Pi_{\text{pr}} - \Pi_{\text{HB}} - RT \sum_{i = \text{sodium, water, and nonurea solutes in RBCs}} \gamma_i (C^i - C_{\text{eq}}^i) \right) \tag{A21}
\]

where \( \Pi_{\text{pr}} \) and \( \Pi_{\text{HB}} \) are the oncotic pressures due to proteins in plasma and to hemoglobin in RBCs, respectively.
The paracellular flux of solute i (i = sodium, protein, urea) across vasa recta walls can be written as

\[ J_i^W (\text{para}) = J_i^P (\text{para}) \times \left( 1 - \sigma_i \right) \frac{C_i^p - C_i^c \exp(-P_e)}{1 - \exp(-P_e)} \]  

(A22)

where \( P_i^W (\text{para}) \) is the permeability of the paracellular pathway to solute i, and the Peclet number, \( P_e \), is a measure of the importance of convection relative to diffusion. For solutes other than urea, \( J_i^P (\text{para}) \) is given by DVR walls and RBC membranes, respectively.

\[ P_i^W (\text{para}) = J_i^P (\text{para}) \times \left( 1 - \sigma_i \right) \]  

(A23)

where \( P_i^W (\text{para}) \) is the permeability of the paracellular pathway to solute i, and the Peclet number, \( P_e \), is a measure of the importance of convection relative to diffusion. For solutes other than urea, \( J_i^P (\text{para}) \) is given by DVR walls and RBC membranes, respectively.

\[ J_i^D = P_i^D (C_i^p - C_i^c) \]  

(A24)

The flux of urea across the RBC membrane is given by

\[ J_i^P = P_i^P (C_i^c - C_i^c) \]  

(A25)

In OMDVR, the plasma-to-pericyte and pericyte-to-interstitium fluxes of NO (see Eqs. 17 and 18) can be written as, respectively

\[ J_i^W = P_i^W (C_i^c - C_i^c) \]  

(A26)

\[ J_i^P = P_i^P (C_i^c - C_i^c) \]  

(A27)

Parameter and initial values, expressions for the cell-wall surface area ratio, the number of vasa recta, the cross-sectional area of the interstitium, the relationship between concentration and oncotic pressure, as well as the numerical methods were given in our previous study (65). Some of the initial values and morphological data are listed in Table 1. The number of collecting ducts and outer mTALs was calculated based on the experimental data of Mejia et al. (40, 41).

We obtained the following regression

\[ N_{\text{cell}}(z) = -4,452.6z^3 + 12,747z^2 - 14,205z + 5,937.3 \]  

(A30)

\[ N_{\text{mTAL}}(z) = -12,766z + 10,297 \]  

(A31)

where z is the dimensionless axial length along the corticomedullary axis.

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GRANTS

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REFERENCES


Nitric oxide in vasa recta


