Free nitric oxide diffusion in the bronchial microcirculation

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Condorelli, Peter, and Steven C. George. Free nitric oxide diffusion in the bronchial microcirculation. Am J Physiol Heart Circ Physiol 283: H2660–H2670, 2002. First published August 22, 2002; 10.1152/ajpheart.00003.2002.—Theoretical mass transfer rates and concentration distributions were determined for transient diffusion of free nitric oxide (NO) generated in vivo from vascular endothelial cells. Our analytical framework is typical of the bronchial circulation in the human pulmonary system but is applicable to the microvascular circulation in general. We characterized mass transfer rates in terms of the fractional mass flux across a boundary relative to the total endothelial NO production rate. NO concentration in the tissue surrounding blood vessels was expressed in terms of fractional soluble guanylate cyclase (sGC) activity. Our results suggest that endothelium-derived free NO is capable of vascular smooth muscle dilation despite its rapid consumption by hemoglobin in blood. An optimal blood vessel radius of 20 μm was estimated for NO signaling. We hypothesize intermittent generation of endothelial NO as a possible mechanism for sGC activation in vascular smooth muscle. This mechanism enhances the efficacy of NO-modulated vascular smooth muscle dilation while minimizing NO losses to blood and surrounding tissue.

ENDOTHELIUM-DERIVED relaxing factor (EDRF), a vasodilator released by arterial endothelial cells, has been identified as either free nitric oxide (NO) or a closely related compound (19). The role of NO as a ubiquitous intracellular messenger is well documented (1, 10, 52). NO is generated in vivo by the enzymatic conversion of L-arginine (L-Arg) to L-citrulline, which is catalyzed by nitric oxide synthase (NOS). The constitutive membrane-bound isoform, endothelial NOS (eNOS), produces NO in vascular endothelial cells, resulting in the dilation of blood vessels. NO activates the soluble isoform of the allosteric enzyme guanylate cyclase (sGC), which catalyzes the conversion of guanosine 5'-triphosphate to cGMP. The subsequent rise in cGMP concentration ultimately results in the dilation of smooth muscle. sGC activity is partially characterized in terms of its apparent Michaelis constant (k\textsubscript{m}) value [the equilibrium NO concentration ([NO]) at which sGC is 50% activated]. Stone and Marletta (44) reported an upper limit of 250 nM for k\textsubscript{m}. However, recent data suggest that k\textsubscript{m} is most likely an order of magnitude lower (~23 nM; Refs. 9, 55). If k\textsubscript{m} is on the order of 23 nM, previous estimates for the effective distance over which NO can influence the activation of sGC (49) need to be reevaluated.

Vaughn et al. (48, 49) demonstrated that the theoretical “effective diffusion distance” (defined as the distance away from its production source within which [NO] exceeds the k\textsubscript{m} of sGC) is strongly dependent on the geometry of its source. Their results suggest that vascular endothelial cells cannot produce free NO at high enough levels to activate sGC in adjacent smooth muscle cells without the protection of an additional cofactor because of the consumption of NO by oxyhemoglobin (Hb) in blood. Their analysis considered a semi-infinite system at steady state, with k\textsubscript{m} = 250 nM.

Expired human breath contains 4–100 ppb NO (14, 42, 43, 50). Exhaled NO has been proposed as a non-invasive biomarker for disease states characterized by inflammation, such as bronchial asthma and allergies (41–43). The potential of endothelium-derived NO to contribute significantly to the levels appearing in expired breath remains to be investigated.

EDRF is hypothesized to be either free NO or NO bound to a protective cofactor (13, 19). On the basis of in vitro data, the half-life of NO within red blood cells is ~1 μs (11, 17, 29). Thus the rapid reaction of NO with Hb present in erythrocytes suggests that other physical or chemical factors are required to activate sGC in smooth muscle cells. If free endothelium-derived NO is EDRF, how does it escape the abyss of Hb in blood vessels to perform its physiological function? One proposed hypothesis is that an erythrocyte-free zone (EFZ) is formed because of the tendency of erythrocytes to migrate away from the blood vessel wall under flow conditions. The EFZ provides a diffusion barrier between erythrocytes and the inner blood vessel wall (5, 29, 30, 48, 49). Recent studies suggest that erythrocytes regulate NO consumption via Hb by means of an intrinsic diffusion barrier at their cell membrane.

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membranes (18, 47). In either case, NO uptake by Hb is limited by diffusion resistance.

We present here a simplified analytical model for small NO-producing blood vessels within the human bronchial circulation, which are bounded by the airway lumen gas space. We consider both steady-state and transient behavior, for a finite geometry, predict NO concentration profiles and diffusion rates at in vivo conditions, and hypothesize possible mechanisms for free NO-modulated smooth muscle dilation. Our goal is to identify potential pathways that may govern the fate and physiological activity of endothelium-derived NO in the human bronchial circulation.

Glossary

\[
\begin{align*}
C & \text{ NO concentration (nM)} \\
C_i & C(t, r = R_i) = \text{NO concentration at surface;} \ i = 1, 2, 3 \ (\text{nM}) \\
C_{i,\text{ss}} & \text{ Steady-state concentration at surface; } i = 1, 2, 3 \ (\text{nM}) \\
C_i^0(\text{x}) & \text{ Initial steady-state NO concentration distribution (nM)} \\
C & \text{ Bulk fluid phase NO concentration (nM)} \\
C_{1,\text{ss}} & C(t \to \infty, y = 1) = \text{steady-state NO concentration at surface; } i = 1 \ (\text{nM}) \\
C_{2,\text{ss}} & C(t \to \infty, y = 2) = \text{steady-state NO concentration at surface; } i = 2 \ (\text{nM}) \\
\frac{\partial C}{\partial y}, i & \text{ at steady state } = C_{1,\text{ss}}[y_1 \ln (y/y_0)] \\
\frac{\partial C}{\partial y}, i & \text{ at steady state } = (f_1 f_2) C_{2,\text{ss}} - (g_1 f_2 f_1) C_{1,\text{ss}} - Q_{2,\text{ss}} \\
D & \text{ Diffusivity of NO within tissue (cm}^2/\text{s or} \ \mu\text{m}^2/\text{s}) \\
F_i & \text{ Known function of } y \text{ and input parameters; } i = 1, 2 \\
f & \text{ Input function for NO concentration at surface; } i = 1, 2 \\
f_i(y) & \text{ Known function of } y \text{ and input parameters; } i = 1, 2 \\
g_i & \text{ Known function of } y \text{ and input parameters; } i = 1, 2 \\
h_i & \text{ Mass transfer coefficient at boundary; } i = 1, 2, 3 \ (\mu\text{m/s}) \\
h_3 & \text{ Mass transfer coefficient between adventitial boundary and external medium} \ (\mu\text{m/s}) \\
H_3 & h_3 R_1/D = \text{dimensionless mass transfer coefficient} \\
J_i & \text{ Mass diffusion flux at boundary } i \ (\mu\text{M} \cdot \mu\text{m} \cdot \text{s}^{-1}) \\
k_1 & \text{ First-order rate constant for NO consumption in pulmonary tissue (s}^{-1}) \\
k_m & \text{ Apparent Michaelis constant for sGC with NO as substrate} \ (\text{nM}) \\
K_m & \text{ Modified Bessel function of the second kind of order } m \\
q_i(\tau) & \text{ Dimensionless NO production rate; } i = 1, 2 \\
Q_i^0 & \text{ Steady-state NO production rate; } i = 1, 2 \\
Q_i^\text{Max} & \text{ Maximum NO production rate; } i = 1, 2 \\
Q_i(\tau) & \text{ Time duration of simultaneous pulse changes in NO production (s)} \\
Q_i^\text{Max} & \text{ Maximum NO production rate; } i = 1, 2 \\
S_i & \text{ Endothelial NO production rate at surface; } i = 1 \ (\text{nM/s}) \\
S^\text{Max}_i & \text{ Maximum NO production rate per unit surface; } i = 1, 2 \ (\mu\text{M} \cdot \mu\text{m} \cdot \text{s}^{-1}) \\
r & \text{ Radial space coordinate (} \mu\text{m}) \\
r_{\text{eff}} & \text{ Effective diffusion radius (} \mu\text{m}) \\
R_0 & \text{ Radius of hypothetical red blood cell core (} \mu\text{m}) \\
R_1 & \text{ Blood vessel radius at inner membrane surface (} \mu\text{m}) \\
R_2 & \text{ Blood vessel radius at outer membrane surface (} \mu\text{m}) \\
R_3 & \text{ Radial distance from blood vessel center to outer adventitial boundary (} \mu\text{m}) \\
R_{\text{NO}}(C) & \text{ NO consumption rate in pulmonary tissue (nM/s) = } k_1 C \ [R_{\text{NO}}(C) = 0 \text{ in EFZ}] \\
S_{\text{NO},i}(t) & \text{ Endothelial NO production rate per unit surface; } i = 1, 2 \ (\mu\text{M} \cdot \mu\text{m} \cdot \text{s}^{-1}) \\
S_{\text{NO},i}^\text{Max} & \text{ Maximum endothelial NO production rate per unit surface; } i = 1, 2 \ (\mu\text{M} \cdot \mu\text{m} \cdot \text{s}^{-1}) \\
V & \text{ Equivalent sGC activity level (cGMP formation rate) at steady state, } V/V_{\text{Max}} \\
V_{\text{Max}} & \text{ Maximum possible cGMP formation rate (nM/s)} \\
V & \text{ Concentration of species in tissue; species = NO, O}_2 \ (\text{nM}) \\
\delta & \text{ Equilibrium distribution coefficient (of NO in EFZ)} \\
\delta C_\infty & \text{ Driving force term (product of } \delta \text{ and } C_\infty \text{) (nM)} \\
\Delta C_i & \text{ Concentration driving force for mass transfer at boundary; } i = 1, 2 \ (\text{nM}) \\
\Delta Q(\tau) & \text{ Fractional flux at surface; } i = 1, 2, 3 \ (\text{nM}) \\
\Delta Q^\text{Max} & \text{ Theile modulus } = k_1 R_1^2/D \\
\eta_i & \text{ Fractional flux at surface; } i = 1, 2, 3 \ (\text{nM}) \\
\kappa^2 & \text{ Time duration of simultaneous pulse changes in NO production (s)} \\
T & \text{ On-time for a continuous (square wave) pulse in NO production (s)} \\
\text{AJP-Heart Circ Physiol} \cdot \text{VOL 283} \cdot \text{DECEMBER 2002} \cdot \text{www.ajpheart.org}
T_2 \quad \text{Off-time for a continuous (square wave) pulse in NO production (s)}

**Subscripts and Superscripts**

0 \quad \text{Initial or basal condition}

i \quad \text{Index corresponding to } r = R_1 (i = 1), r = R_0 (i = 2), \text{and } r = R_3 (i = 3) \text{ or general integer}

i+ \quad \text{Evaluation at the outer surface of a boundary}

i− \quad \text{Evaluation at the inner surface of a boundary}

j \quad \text{Index corresponding to general integer; } j = 1, 2, 3

Max \quad \text{Maximum}

ss \quad \text{Steady state}

**METHODS OF ANALYSIS**

**Endothelial NO production near an arteriole.** A typical geometry for a blood vessel of the bronchial circulation is depicted in Fig. 1, with the radial coordinate denoted by r. Erythrocyte(s) are assumed to be clustered at the center of the blood vessel (r < R_0). R_1 and R_0 denote the radii of the blood vessel and its hypothetical red blood cell (RBC) core, respectively, with the region R_5 < r < R_3 comprising an EFZ.

For small blood vessels, individual endothelial cells form nearly cylindrical annuli over small axial and angular distances. eNOS is a membrane-bound enzyme (54). We assume that NO is produced at the endothelial membranes, which are thin compared with the total cell thickness (see Fig. 1B). The NO production rates, per unit surface, at the inner (r = R_1) and outer (r = R_2) endothelial cell membranes are $S_{NO,1}$ and $S_{NO,2}$, respectively (49). Because the substrate, L-Arg, is a ubiquitous amino acid, we assume that $[\text{L-Arg}] > [\text{NO}]$. Thus $S_{NO,1}$ and $S_{NO,2}$ are independent of [NO]. Recent experimental data suggest that micromolar levels of NO reversibly inhibit NOS (39). However, these levels are at least two orders of magnitude higher than those considered here.

**Governing equation and boundary conditions.** We assume that, over limited regions of the blood vessel circumference, angular and axial diffusion rates are small compared with the radial diffusion rate. Hence, the one-dimensional diffusion equation in cylindrical coordinates applies to each region

$$
\frac{\partial C}{\partial t} = \frac{D}{r} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) - R_{NO}(C)
$$

where t is time, r is the radial coordinate, C is the concentration of NO, $R_{NO}(C)$ is the NO consumption rate per unit volume, and $D$ is the diffusivity of NO within tissue. $D$ is estimated as the diffusivity of NO within water (i.e., $D = 3.3 \times 10^{-5}$ cm$^2$/s) (25, 26, 48).

The most active scavengers of NO within pulmonary tissue are most likely $O_2$ and Hb (1, 14, 40, 51, 52). The reaction of NO with $O_2$ is first order with respect to [NO] and occurs in the adventitial tissue, whereas its consumption by Hb occurs in the RBC core. NO consumption rates are assumed to be negligible within erythrocyte-free blood plasma, where Hb and $O_2$ are absent [$R_{NO}(C) = 0$ in the EFZ]. However, irreversible oxidation of NO in RBCs is assumed to be instantaneous within the RBC core (i.e., at $r = R_0$, C(t, r = R_0) = 0). We assume first-order NO consumption within the pulmonary tissue surrounding the blood vessel, $R_{NO}(C) = k_1 C$, where $k_1$ is the first-order rate constant with respect to NO. Equation 1 can be expressed in terms of dimensionless length, $y = r/R_1$, and time, $\tau = Dt/R_1^2$ (see APPENDIX). The Thiele modulus, $\kappa^2 = k_1 R_0^2/D$, is also dimensionless and corresponds to the ratio of reaction rate to diffusion rate.

The NO concentrations on each side of a boundary between adjacent tissue regions are assumed equal. However, at the inner and outer endothelial membrane surfaces ($r = R_1$ and $r = R_2$), NO production, $S_{NO,i}(t)$, contributes to the molar diffusion flux, $J_i = (-D\partial C/\partial r)_i$, as expressed by the internal boundary condition

$$J_{i+} - J_{i−} = S_{NO,i}(t) \quad \text{at } r = R_1 (i = 1) \text{ and } r = R_2 (i = 2) \quad (2)$$

where the molar diffusion flux is directed outward from the NO-producing cell(s) and the subscripts i+ and i− denote evaluation of the molar diffusion flux, $J_i$, at the outer and inner membrane surfaces, respectively (i.e., $r = R_1$ and $r = R_1^−$ or $r = R_2^+\text{ and } r = R_2$). If the NO production rate per unit surface, $S_{NO,i}(t)$, in Eq. 2 is zero, the concentration gradient is continuous across the boundary. The relationships implied by Eq. 2 are based on our assumption that the cell membrane is infinitesimally thin (see Fig. 1B).

Non-NO-producing adventitial tissue lies external to the cell membrane boundaries. We assume that the outer adventi-
The boundary condition for steady-state analysis is:

\( J_3 = -D{\partial C_3/\partial r}\big|_{r_3} = h_3(C_3 - \delta C) \quad \text{at} \quad r = r_3 \) (3)

where \( \delta \) is the equilibrium distribution coefficient.

\( \gamma_C \) is the bulk fluid concentration (assumed constant), \( h_3 \) is the mass transfer coefficient between the adventitial boundary (2) and the external medium (\( H_3 = h_3 R_3/D \)) in dimensionless form, and \( [C_3 - \delta C] \) represents a driving force term.

For fractional mass transfer flux, we define the fractional flux, \( \eta_i \), as the mass flux across a boundary \( i \) relative to the total endothelial NO production rate. For a cylindrically shaped blood vessel, where \( S_{NO,i} \) is uniform over each membrane surface \((i = 1, 2)\)

\[
\eta_i = \frac{|(-\partial C_i/\partial r)|_{r_3}}{S_{NO,i}R_i + S_{NO,2}R_2} = \frac{|(-\partial C_i/\partial r)|_{r_3}}{Q_1 + Q_2 y_2} = 1 - \frac{1}{2} + \frac{3}{2} \quad \text{(4)}
\]

where \( y_i = R_i R_3 \) \((i = 1, 2, 3)\) and \( Q_i = S_{NO,i} R_i / D \) \((i = 1, 2)\) denote the scaled production rates. Equation 4 assumes that \( J_i \) is directed normal to surface \( i \) and away from the endothelial cell. If the diffusive flux is directed into the endothelial cell, \( \eta_i < 0 \). There is no accumulation of NO within the endothelial cell at steady state; therefore \( \eta_1 + \eta_2 + \eta_3 = 0 \). At the outer boundary of the adventitial region \((i = 3)\) and the internal boundary \((r = R_3)\), computation of \( \eta_3 \) from Eq. 4 is valid over a limited region of the blood vessel circumference, where the angular flux contribution is negligible. Henceforth, we denote \( \eta_1, \eta_2, \) and \( \eta_3 \) as \( \eta_1, \eta_2, \) and \( \eta_3 \) or \( (i = 1, 2, 3) \), respectively.

Steady-state analysis. When both the external conditions and the NO production rates remain unchanged for a long time, the time derivative in Eq. 1 vanishes and the steady-state solution can be derived by integration of the resulting ordinary differential equation (see APPENDIX). Although this yields a complex system of algebraic expressions, values of \( \eta_i \) are readily computed from Eq. 4.

At steady state, \( \eta_i \) \((i = 1, 2, 3)\) values depend on seven specified parameters \( k_1, y_0 = R/R_1, y_2 = R_2/R_1, y_3 = R_3/R_1, R_1, \delta C, \) and \( h_3 \) but are independent of the NO production rates. Also, at steady state, there is no accumulation of NO in the endothelium and \( \eta_1 + \eta_2 = 1 \). The total flux entering both blood and surrounding tissue equals the NO production rate within the endothelium. Thus only \( \eta_3 \) needs to be considered in this analysis, and we treat \( \eta_i \) \((i = 2, 3)\) as output parameters dependent on the specified \( (input) \) parameters, which are treated as independent variables. The mean values and ranges of the input parameters are summarized in Table 1. We estimated EF2 thickness, \( R_1 - R_0 \), and representative probability distributions on the basis of experimental measurements of vascular hematocrit as a function of blood vessel radius at typical blood velocities \( (4, 27, 48, 49) \). Thus these estimates account for the Fahraeus effect. We assumed NO production rates of \( S_{NO,1} \) and \( S_{NO,2} \) to be 26.5 \( \mu M \cdot \mu m \cdot s^{-1} \) for this analysis, on the basis of published data \( (33, 48) \). Because, at steady state, \( \eta_0 \) and \( \eta_2 \) are dependent only on the ratio \( S_{NO,1} \) and \( S_{NO,2} \) and independent of the magnitude of NO production, we did not study \( S_{NO,1} \) and \( S_{NO,2} \) as input parameters.

### Table 1. Input parameter ranges for steady-state regression analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Lower Limit</th>
<th>Mean Value</th>
<th>Upper Limit</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>s^{-1}</td>
<td>0.05</td>
<td>0.35</td>
<td>1.5</td>
<td>14, 25, 33, 53</td>
</tr>
<tr>
<td>( y_0 )</td>
<td></td>
<td>0.70</td>
<td>0.80</td>
<td>0.95</td>
<td>4, 27, 48, 49</td>
</tr>
<tr>
<td>( y_2 )</td>
<td></td>
<td>1.05</td>
<td>1.25</td>
<td>1.5</td>
<td>4, 48, 49</td>
</tr>
<tr>
<td>( y_3 )</td>
<td></td>
<td>2</td>
<td>6</td>
<td>20</td>
<td>4, 12, 28</td>
</tr>
<tr>
<td>( R_1 )</td>
<td>( \mu m )</td>
<td>3</td>
<td>10</td>
<td>20</td>
<td>6, 28</td>
</tr>
<tr>
<td>( \delta C )</td>
<td></td>
<td></td>
<td>0.16</td>
<td>0.7</td>
<td>46</td>
</tr>
<tr>
<td>( h_3 )</td>
<td>( \mu m / \pi )</td>
<td>3 \times 10^4</td>
<td>1 \times 10^6</td>
<td>6 \times 10^5</td>
<td>2, 8</td>
</tr>
</tbody>
</table>

*Based on 0.5 to 15 s half-life (14, 25, 33, 53); \( b_1 = 0.95 \) (large blood vessels) \( (48, 49) \), to 0.7 (capillaries of 10-μm diameter corresponding to 50% hematocrit) \( (4, 27) \); endothelium thickness, \( R_2 - R_1 \), range: 0.2 \( \mu m \) for capillaries \((5)\) to 10 \( \mu m \) for large blood vessels \((48, 49) \); based on conductive airway epithelial thickness with generation number \((4, 8, 12, 28) \); based on morphological data for the bronchial circulation \((6, 28) \); based on endogenous NO concentrations predicted in conductive airway lumen \((46) \); based on theoretical mass transfer coefficients predicted for the human conductive airways \((2, 8) \). See Glossary for definitions.

To simplify the complex form of the steady-state solution, the dependence of \( \eta_i \) \((i = 2, 3)\) on the specified parameters is assessed by linear regression analysis, with input parameter values selected by Latin hypercube sampling \((LHS) \) \((34) \). Thus we correlate \( \eta_i \) to the form \( \eta_i = \beta_i \eta_{x_1} \), where \( x_1 \) and \( \beta_i \) denote the input parameters and sensitivity coefficients of the output, \( \eta_i \), respectively. The purpose of this analysis is to identify trends, which represent the dependence of \( \eta_i \) on the input parameter values specified in the simulations. \( P \) values, which correspond to the probabilities that either \( \eta_i \) is independent of a particular \( x_1 \), provide a quantitative assessment of the significance of the dependence of \( \eta_i \) on each input parameter. Thus we calculate rigorous results with our relatively complex mathematical model and attempt to identify the most important input parameters with regression analysis.

**Transient analysis.** The transient solution of Eq. 1 is obtained by the method of separation of variables, which leads to an infinite series of Bessel functions in the radial space \((7, 15, 38) \). The solution is expressed as the sum of steady-state and transient parts \((see \text{APPENDIX}) \). The initial steady-state concentration distribution corresponds to the basal NO production rates, \( S_{NO,i} \) \((i = 1, 2)\). Transient computations consider abrupt changes in \( S_{NO,i} \) to their maximum values, \( S_{Max,i} \) at time \( t = 0 \). Identical changes in \( S_{NO,i}(t) \) are assumed to occur simultaneously for \( i = 1 \) and \( i = 2 \). Maximum NO production rates are estimated as \( S_{Max,1} = S_{Max,2} = 26.5 \mu M \cdot \mu m \cdot s^{-1} \), based on published data \( (33, 48) \). Basal NO production rates are estimated as the steady-state levels required to maintain \( NO \) \((0.2 \text{ nM (1 sCG activation)) within the vascular smooth muscle (} S_{NO,1} = S_{NO,2} = 0.1 \mu M \cdot \mu m \cdot s^{-1} \)). The transient solution is expressed in terms of the dimensionless NO production rates, \( q(t) = (S_{NO,1}(t) - S_{NO,1})/S_{Max,1} + S_{NO,2} - S_{NO,1} - S_{NO,2} \), which may be arbitrary functions of time \((15) \). The maximum values of \( q(t) \) satisfy \( q_{Max,1} + q_{Max,2} = 1 \).

Three NO production scenarios are considered. The “step change” scenario considers a single abrupt simultaneous change in \( S_{NO,i}(t) \) from \( S_{NO,i} \) to \( S_{Max,i} \) at \( t = 0 \). The “single pulse change” scenario considers a single abrupt simultaneous change in \( S_{NO,i}(t) \) from \( S_{NO,i} \) to \( S_{Max,i} \) at \( t = 0 \) and from \( S_{Max,i} \) to \( S_{NO,i} \) at \( T = T \) (pulse duration time). In the “continuous pulse” (square wave) scenario, \( S_{NO,i}(t) \) values are maintained at \( S_{Max,i} \) and \( S_{NO,i} \) over time intervals \( T_1 \) and \( T_2 \),
respectively, with abrupt changes between these two extremes to form a square-wave pattern with this cycle repeating indefinitely. The time intervals $T_1$ and $T_2$ are referred to as the pulse “on-time” and “off-time,” respectively. The transient response for this scenario is evaluated after a relatively long time ($t > 20$ s). $T_1$ and $T_2$ were selected as 500 ms and 5 s, respectively, on the basis of previously published experimental data (22, 33, 36, 55) and previously published simulations of these data (9, 24–26, 48, 49, 53). These data and simulations demonstrated that sGC activation by NO is at least an order of magnitude faster than its deactivation via NO dissociation (i.e., $5 \text{s} / 500 \text{ms} = 10$) and suggested that NO may exert its physiological influence within seconds of its generation.

We define the effective diffusion radius, $r_{\text{eff}}$, as the radial distance away from the blood vessel within which [NO] exceeds the $k_m$ value corresponding to 50% sGC activation (49). These results are expressed as the scaled effective diffusion radius, $y_{\text{eff}} = r_{\text{eff}}/R_1$. We convert [NO] to equivalent relative sGC activity level at steady state, $V/V_{\text{Max}}$, on the basis of previous work, which determined a $k_m$ of $\sim 25$ nM and a Hill coefficient of 1.3 (9, 55). Thus basal (1%), 10%, 50%, and 90% sGC activity levels are assumed at [NO] = 0.2, 4, 22, and 100 nM, respectively.

RESULTS

Steady-state analysis. Table 2 summarizes the results of LHS linear regression analysis in terms of the $P$ values and regression coefficients, which are indices of the significance and sensitivity, respectively, with respect to the corresponding input parameters. Both $\eta_2$ and $\eta_3$ are more sensitive to $y_0$, $y_2$, and $y_3$ than the other parameters, as demonstrated by their low $P$ values and high regression coefficients (see Table 2); $\eta_2$ is weakly dependent on both $R_1$ and $k_1$. Unlike $\eta_2$, $\eta_3$ decreases with $k_1$ and is not significantly dependent on $R_1$. The dependence of $\eta_2$ on $y_0$, $y_2$, and $y_3$ with $R_1 = 10$ μm is depicted in Fig. 2. The results shown in Fig. 2 are consistent with the regression coefficients shown in Table 2 ($\eta_2$ increases with $y_2$, decreases with $y_0$, and decreases with $y_3$ at fixed $y_0$). Although $\eta_2$ increases with both $R_1$ and $k_1$ (see Table 2), this subtle dependence is not depicted in Fig. 2. The dependence of $\eta_3$ on the same input parameters (see Fig. 3) shows behavior analogous to that of $\eta_2$. As a result of chemical consumption, $\eta_3 < \eta_2$ for fixed values of the input parameters (compare Figs. 2 and 3).

Over the range of input parameter values considered in this study, computed values of $\eta_3$ and $\eta_2$ varied between 0.01 and 0.9 (data simulations not shown). However, as depicted in Figs. 2 and 3, an upper limit of $0.4–0.6$ for $\eta_3$ is probably more realistic under most scenarios of physiological interest. For an arteriole, which is located very close to the airway lumen, this suggests that, at most, 40–60% of the produced NO will reach the air space. We emphasize that this may still be negligible compared with the contributions of other NO-producing tissue, such as the bronchial epithelium.

The steady-state dependence of the dimensionless effective diffusion radius, $y_{\text{eff}} = r_{\text{eff}}/R_1$, on the blood vessel radius, $R_1$, is shown in Fig. 4. In this analysis, the other input parameters were fixed at their mean values (see Table 1). $y_{\text{eff}}$ goes through a maximum of approximately five blood vessel radii at $R_1 = 27$ μm (see Fig. 4). This result is consistent with previous work, which predicts maximum effective diffusion distances at microvessel diameters of $30–100$ μm (49). We selected the nominal value, $R_1 = 20$ μm, as the near-optimal blood vessel radius for our transient analysis. This size is within the observed range for blood vessels in the bronchial circulation (4, 6, 28).

Transient analysis. The results presented in Figs. 5–7 were computed with all input parameters set at their mean values (see Table 1) and the airway lumen outer adventitial boundary condition, unless otherwise indicated. We express transient NO concentration profiles in terms of equivalent relative sGC activity level at steady state, $V/V_{\text{Max}}$. For step changes in the NO production at $R_1 = 5$ and 20 μm, the dependence of $V/V_{\text{Max}}$ on $R_1$ is significant (compare Fig. 5, A and B). Figure 5 shows two $V/V_{\text{Max}}$ peaks for both 0.01 and 0.1 ms (Fig. 5A; $R_1 = 5$ μm) and 0.1 and 1 ms (Fig. 5B; $R_1 = 20$ μm). These peaks correspond to the two NO sources at the inner and outer endothelial membranes and have a “Gaussian” appearance, because at these small times very little NO has reached the RBC core and outer adventitial boundary sinks. Within 50 ms, sGC activity levels in the vascular smooth muscle region reach 45–50% ($r \approx 6–7$ μm) for $R_1 = 5$ μm (Fig. 5A) and 70–80% ($r \approx 24–28$ μm) for $R_1 = 20$ μm (Fig. 5B). Because sGC activity level is dependent on S$_\text{NO}$, this analysis should be revisited when more information pertaining to in vivo NO production rates becomes available.

Figure 6 depicts the time dependence of the fractional fluxes with $R_1 = 20$ μm. The other input param-

Table 2. Steady-state regression analysis from Latin hypercube sampling

<table>
<thead>
<tr>
<th>Input Parameter</th>
<th>Units</th>
<th>Range</th>
<th>$P$ value</th>
<th>Coefficient ($\beta/\eta_2$)</th>
<th>$P$ value</th>
<th>Coefficient ($\beta/\eta_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>s$^{-1}$</td>
<td>0.05–1.5</td>
<td>$3.0 \times 10^{-3}$</td>
<td>0.009</td>
<td>$7.4 \times 10^{-6}$</td>
<td>-0.016</td>
</tr>
<tr>
<td>$y_0$</td>
<td>μm</td>
<td>0.70–0.95</td>
<td>$1.5 \times 10^{-49}$</td>
<td>-0.571</td>
<td>$1.0 \times 10^{-41}$</td>
<td>-0.523</td>
</tr>
<tr>
<td>$y_2$</td>
<td>μm</td>
<td>1.05–1.5</td>
<td>$7.2 \times 10^{-36}$</td>
<td>0.256</td>
<td>$2.6 \times 10^{-28}$</td>
<td>0.234</td>
</tr>
<tr>
<td>$y_3$</td>
<td>μm</td>
<td>2–20</td>
<td>$7.0 \times 10^{-32}$</td>
<td>-0.005</td>
<td>$1.2 \times 10^{-40}$</td>
<td>-0.008</td>
</tr>
<tr>
<td>$R_1$</td>
<td>μm</td>
<td>3–20</td>
<td>$5.2 \times 10^{-3}$</td>
<td>0.003</td>
<td>0.57</td>
<td>-0.001</td>
</tr>
<tr>
<td>$\delta C_\text{a}$</td>
<td>nM</td>
<td>0–0.7</td>
<td>0.48</td>
<td>-0.005</td>
<td>0.22</td>
<td>-0.010</td>
</tr>
<tr>
<td>$h_3$</td>
<td>μm/s</td>
<td>$3 \times 10^{-6}–6 \times 10^6$</td>
<td>0.51</td>
<td>$5 \times 10^{-10}$</td>
<td>0.34</td>
<td>$8 \times 10^{-10}$</td>
</tr>
</tbody>
</table>

See Table 1 for references and Glossary for definitions.
eters were fixed at their mean values (see Table 1). For \( t < 200 \text{ ms} \), \( \eta_3 \) is very small, but for \( t > 2 \text{ s} \), \( \eta_3 \rightarrow \eta_2 \). Hence, ~100 ms is required for the NO signal to reach the outer adventitial boundary. For \( t < 10 \text{ ms} \), \( \eta_1 < \eta_2 \) and \( \eta_1 + \eta_2 < 1 \), whereas for \( t > 2 \text{ s} \), \( \eta_1 \rightarrow 0.83 > \eta_2 \rightarrow 0.16 \) and \( \eta_1 + \eta_2 \rightarrow 1 \). This behavior results from the combined effects of NO accumulation in the endothelium and the finite transit time required for NO diffusion from the inner wall of the blood vessel to the RBC core.

For a step change in NO production with \( R_1 = 20 \text{ µm} \), \( V/V_{\text{Max}} \) reaches 85–90% equivalent (steady state) sGC activity in the vascular smooth muscle region (\( r^{c_s} = 24–28 \text{ µm} \)) within 500 ms (Fig. 5B). This implies that if [NO] is maintained at the levels achieved after 500 ms, sGC present in vascular smooth muscle will eventually reach 85–90% of its maximum activity. Figure 7 compares \( V/V_{\text{Max}} \) for pulses of the same amplitude and duration with \( R_1 = 20 \text{ µm} \). For a single pulse of duration, \( T = 500 \text{ ms} \) (see Fig. 7), the same activity level is achieved. After the pulse is “turned off” at \( t = 500 \text{ ms} \), \( V/V_{\text{Max}} \) drops to near-basal levels within 5 s. The transient responses for continuous (square wave) pulses with an on-time of \( T_1 = 500 \text{ ms} \) and an off-time of \( T_2 = 5 \text{ s} \) were virtually identical to those depicted in Fig. 7 for a single pulse (data simulations not shown). Thus the results shown in Fig. 7 apply to both the single-pulse and square-wave pulse scenarios.

**Fig. 2.** Fractional flux \( \eta_2 \) at the outer blood vessel surface with inner blood vessel radius (\( R_1 \)) = 10 µm, mass transfer coefficient (\( h_3 \)) = \( 1 \times 10^6 \text{ µm/s} \), and driving force term (\( \delta C_3 \)) = 0.16 nM.

**Fig. 3.** Fractional flux \( \eta_3 \) at the airway lumen boundary with \( R_1 = 10 \text{ µm} \), \( h_3 = 1 \times 10^6 \text{ µm/s} \), and \( \delta C_3 = 0.16 \text{ nM} \). \( y \), Dimensionless radial space coordinate.

**Fig. 4.** Effective diffusion distance, \( y_{\text{eff}} \), at steady state.

**Fig. 5.** Radial profiles of soluble guanylate cyclase (sGC) activity, \( V/V_{\text{Max}} \), with time, \( t \), for a step increase in nitric oxide (NO) production rate. \( \eta_1, \eta_0 = R_0/R_1, \eta_2 = R_2/R_1, \eta_3 = R_3/R_1, h_3 = 1 \times 10^6 \text{ µm/s} \), and \( \delta C_3 \) are at the mean values listed in Table 1, and the maximum NO production rates are \( S_{\text{NO,1}}^{\text{Max}} = S_{\text{NO,2}}^{\text{Max}} = 26.5 \text{ µM µm s}^{-1} \). A: \( R_1 = 5 \text{ µm} \). B: \( R_1 = 20 \text{ µm} \).
DISCUSSION

We have computed theoretical concentration profiles and diffusion rates around NO-producing blood vessels based on a mathematical model applicable to the human pulmonary system. Our results confirm that endothelium-derived free NO is capable of modulating vascular smooth muscle tone by activation of sGC. We hypothesize that intermittent generation of NO by eNOS may minimize losses to blood and surrounding tissue. In addition, we cannot rule out a potential contribution of eNOS to the levels of NO appearing in expired breath.

Several mathematical models have been used to study in vivo NO diffusion. The point source model (24–26, 53) assumes that the physical dimensions of each source (NO-producing cell) are small compared with the surrounding medium and sums up all contributions from multiple sources. For a single point source, which generates NO for 1–10 s, this model predicts that the "physiological sphere of influence" is ~200 μm away from its source, provided the half-life of NO within tissue is <5 s. These results led to the hypothesis that, with the exception of blood, chemical consumption of NO in most tissues is slow compared with its diffusion rates.

Vaughn et al. (48, 49) estimated effective diffusion distances for endothelial NO production in blood vessels. Their model accounted for the finite geometry of the production source. However, their analysis was limited to the steady-state behavior of a semi-infinite system, and they assumed $k_m = 250$ nM for sGC activation (44). Their results demonstrate the significant influence of blood vessel geometry and rapid binding of NO to Hb in blood. They suggested that free NO produced by vascular endothelial cell(s) cannot escape consumption by Hb and still activate sGC in vascular smooth muscle cells without the protection of an additional cofactor.

Under flow conditions, NO consumption by erythrocytes proceeds at a slower rate than that observed in the presence of free Hb (29, 47). Proposed explanations for this slower consumption rate include the diffusion resistance of the EFZ (devoid of erythrocytes) around the RBC core (30) and formation of relatively stagnant (unstirred) plasma layers around individual erythrocytes within the RBC core (32). Alternately, the possibility that a cytoskeletal network of proteins adjacent to the cell membranes of erythrocytes provides additional resistance to NO diffusion has also been proposed (18). Each of the above hypotheses suggests that an additional diffusion barrier limits consumption of NO by erythrocytes. Our model approximates this additional mass transfer resistance as an annular ring of erythrocyte-free plasma around a central RBC core.

Recent evidence suggests that $k_m \approx 23$ nM, an order of magnitude lower than the upper limit of 250 nM reported by Stone and Marletta (Ref. 44; see Refs. 9, 55). On the basis of these data, we expressed NO concentrations in terms of sGC activity level, $V/V_{\text{Max}}$ (Figs. 5 and 7), and reevaluated the effective diffusion distance at steady state (Fig. 4). The incorporation of a finite external boundary provides additional impetus for mass transport away from the blood vessel. Although our model assumes zero [NO] at the outer boundary of a central RBC core, we include additional diffusion resistance resulting from the EFZ thickness, which is consistent with current experimental data. Our results suggest that the EFZ substantially limits NO consumption by Hb and is potentially a major contributor to the effectiveness of free NO as a vasodilator.

Our results suggest that the above considerations are sufficient for free NO to perform its physiological role of vasodilation in smooth muscle. However, although 45–80% of full sGC activity is achieved in vascular smooth muscle at steady state, ~80% of the produced NO diffuses into the blood vessel (see Figs. 5 and 6). The time dependence of NO production provides a potential mechanism for enhanced utilization of NO for smooth muscle dilation (Figs. 6 and 7). At short times, these transient concentration profiles have Gaussian shapes as NO accumulates within the endothelium. Under these conditions, large concentra-
tion changes take place over a very thin region. Thus initial NO diffusion rates, both into and away from the blood vessel, are roughly equal and are virtually unaffected by external boundaries (Figs. 5 and 6). Transient concentration profiles strongly depend on the blood vessel radius, $R_1$ (Fig. 5). For a near-optimal blood vessel radius, $R_1 = 20 \mu m$ and $S_{NO,1} = S_{NO,2} = 26.5 \mu M \cdot \mu m^{-1} \cdot s^{-1}$ (48), 60–80% of full sGC activation can be achieved in vascular smooth muscle within 50–100 ms (Fig. 5B).

With $R_1 = 20$, we compared $\nu / \nu_{Max}$ for step, single-pulse, and continuous (square wave) pulse changes in NO production rate. Our results show that pulsatile generation of NO via eNOS results in enhanced utilization of NO for smooth muscle dilation. Square-wave and single-pulse changes in NO production achieve sGC activity levels of amplitude equal to that of the corresponding step change case (compare Figs. 5B and 7). However, the weight-averaged NO production rate for the square-wave case is only 10% of that for the corresponding step change case. The enhanced utilization efficiency results from reduced NO losses to Hb in the blood vessel.

Analysis of in vitro data demonstrates that activation of sGC by NO is rapid compared with its NO dissociation from sGC. Dissociation proceeds with a half-life of ~1–2 min (3, 9, 19, 22, 23, 36). However, sGC activation is at least an order of magnitude faster. Despite limited capacity, the initial binding rate of NO to sGC in smooth muscle is nearly as rapid as its binding rate with Hb in blood (9, 55). Thus NO binds to sGC, present in smooth muscle (at high [NO]) with maximum NO production rate, $S_{NO,2}$, much faster than it dissociates (at low [NO] with basal NO production, $S_{NO,1}$). Therefore, if NO is generated in brief, intermittent bursts, diffusion rates away from the source are virtually identical in both directions and comparable amounts of NO bind to both sGC and Hb. When [NO] is high, sGC is rapidly converted into the activated state. However, when NO production is “turned off”, [NO] decreases, but dissociation is so slow that most of the sGC remains in the activated state and does not approach the basal state for 1–2 min. Our model results depict a scenario in which $\nu / \nu_{Max}$ reaches ~80% within 500 ms and the next “pulse” of NO production reaches adjacent smooth muscle 5 s later, thereby maintaining sGC in the activated state. Thus $T_1 = 500$ ms and $T_2 = 5$ s represent a near-optimal NO utilization efficiency.

Temporal changes in shear stress and circumferential strain typically occur over time scales on the order of 1 s. These changes impose dynamic forces on vascular endothelial cells and impact NO production rates (35, 37). Therefore, cyclic changes in vascular stress and strain could trigger endothelial NO production according to the scenario described above. In addition, muscular contraction and relaxation have been shown to exhibit cyclic behavior with periods of ~1 s (21). Because NO remains bound to sGC for at least several seconds, a periodic NO signal would eventually force sGC into the activated state. Therefore, we hypothesize that pulsatile changes in blood flow actuate bursts of NO production over time scales on the order of seconds, which are capable of essentially full sGC activation.

At steady state, the fractional fluxes at the endothelial membranes are dependent on the endothelial and EFZ thicknesses (see Table 2 and Fig. 2). If the distance of the blood vessel from the outer adventitial boundary (characterized by $y_3$ and the blood vessel radius) is large, $\eta_2$ is also dependent on $k_1$ but essentially independent of $y_3$. Conversely, if the distance from the adventitial boundary is small, this dependence reverses ($\eta_2$ is independent of $k_1$ but dependent on $y_3$). At fixed $y_3$, as $R_1$ increases the distance from the outer adventitial boundary, $R_3 = y_3 R_1$, also increases. Hence, the further the blood vessel is from the outer adventitial boundary, the more time a diffusing molecule of NO has to react with scavengers in pulmonary tissue. As $R_3$ increases, increased NO consumption in the adventitial region results in a steeper concentration gradient and therefore a higher molar flux at the source ($r = R_2$), which leads to a higher value for $\eta_3$. Thus, for small values of $R_1$, $\eta_2$ is more sensitive to $y_0$, $y_2$, and $y_3$ than the rate constant, $k_1$. As $R_1$ increases the dependence of $\eta_2$ on $k_1$ becomes more significant, and $\eta_2$ becomes independent of $y_3$ as $R_3$ becomes large.

The molar flux estimated at the external boundary (characterized by $y_3$) is lower than the flux determined at the generating source (characterized by $\eta_2$). In contrast to $\eta_2$, $\eta_3$ is essentially independent of $R_1$ and exhibits greater dependence on $k_1$ than $\eta_2$. In addition, as $k_1$ increases, $\eta_3$ increases whereas $\eta_3$ decreases with increased adventitial NO consumption (compare $P$ values and regression coefficients of $7.4 \times 10^{-6}$ and $-0.016$ for $\eta_3$ vs. $3.0 \times 10^{-3}$ and 0.009 for $\eta_2$, respectively, in Table 2). Thus NO consumption enhances mass transport at the production source ($r = R_2$) and attenuates mass transport at the outer adventitial boundary ($r = R_3$). Hence, as one moves further from the NO source, both the NO concentration and the molar flux become attenuated as a result of NO consumption by scavengers.

Table 2 also shows that both $\eta_2$ and $\eta_3$ are very sensitive to $y_0$, $y_2$, and $y_3$ (i.e., the ratios $R_0/R_1$, $R_3/R_1$, and $R_3/R_2$, respectively) but not appreciably sensitive to $R_1$. Hence, $\eta_2$ and $\eta_3$ are determined primarily by the relative differences $1 - y_0 = (R_1 - R_0)/R_1$, $y_2 - 1 = (R_2 - R_1)/R_1$, and $y_3 - 1 = (R_3 - R_1)/R_1$, which define the geometry of the EFZ, rather than by the luminal radius of the vessel. Thus the relative distances (and therefore the mass transfer resistances) between the NO production sources (at $r = R_1$ and $R_2$, respectively) and their closest sinks (at $r = R_0$ and $R_3$, respectively) control fluxes $\eta_2$ and $\eta_3$.

For most of our simulations, inner blood vessel radius, $R_1$, is large relative to the differences $R_1 - R_0$ and $R_2 - R_0$. Therefore, near the blood vessel, the effects of curvature are small and it can be modeled as if it were two flat plates. Thus $\eta_2$ is weakly dependent on $R_1$ and is controlled almost exclusively by $y_0$, $y_2$, and $y_3$. At the outer adventitial boundary ($r = R_3$), this “flat plate approximation” is not valid. However, for most of our
simulations, \( R_3 > R_1 \), and the blood vessel looks like a “point source” of NO. Thus \( \eta_2 \) is very insensitive to \( R_1 \) and is controlled almost exclusively by \( y_0, y_2, \) and \( y_3 \).

As one moves away from the blood vessel, angular and axial diffusion fluxes become more important. Thus the accuracy of the predicted molar flux at the outer adventitial boundary is limited. However, this model does provide an estimate for the maximum possible flux over limited regions of a blood vessel’s circumference. On this basis, blood vessels must be relatively close to the airway lumen to contribute significant amounts of NO to the airway lumen gas space. For example, with \( y_3 = 2 \) (i.e., within 2 blood vessel radii of the airway lumen), \( \eta_3 \) is in the range 0.2–0.65 for \( y_2 = 1.2 \) (see Fig. 3). However, if the blood vessel is far away from the airway lumen, \( \eta_3 \) is much lower (e.g., with \( y_3 = 20 \), typical of the upper conductive Airways of the lungs, \( \eta_3 < 0.2 \); see Fig. 3). Therefore, our results do not contradict the prevailing hypothesis that blood vessels do not contribute significantly to endogenous exhaled NO.

For the typical conditions assumed here, optimal NO signaling efficacy is anticipated for a blood vessel radius of \( 20–30 \mu m \) (Fig. 4). The optimal blood vessel radius represents a dynamic balance between in vivo chemical consumption and NO production rates. For similar geometries, with the dimensionless length parameters, \( y_1 = R/R_1 \), constant, the influence of chemical consumption and NO production increase with \( k_3 = k_1 R_3^2/D \) and \( Q_1 = S_{NO}/R_1/D \), respectively. Thus the optimal radius corresponds to a critical blood vessel geometry at which these two opposing effects balance each other.

Thomas et al. (45) report that the half-life for NO surrounding a vessel is inversely proportional to \( O_2 \) concentration, which implies that NO consumption via its reaction with \( O_2 \) is first-order with respect to \( [O_2] \). Because \([O_2] \) decreases with distance from an arteriole, a gradient (corresponding decrease) in NO consumption rate with distance from the vessel is created. Because this reaction rate is second order with respect to \([NO] \), it would be higher at high \([NO] \) (and lower at low \([NO] \)) than the rate that would be predicted by a first-order mechanism. Thus we expect the apparent first-order constant for NO consumption to decrease as we move away from an arteriole, as proposed by Thomas et al. (45). This implies that the NO consumption rate close to the blood vessel would be faster than that predicted by a first-order mechanism and, conversely, slower as we move away from the blood vessel. This effect would “flatten” the \([NO] \) profile, thereby decreasing the diffusion rate away from the blood vessel. The rate of NO consumption via \( O_2 \) in pure water is very slow (1), and we expect its impact to be modest. However, NO consumption via \( O_2 \) may be accelerated within the hydrophobic interiors of neighboring cell membranes (31).

In conclusion, the rapid rate of reaction of free NO with \( Hb \) in blood does not prevent its action as an important signaling molecule for vascular smooth muscle dilation. Steady-state \( sGC \) activities of 45–80% are achieved in vascular smooth muscle adjacent to 20- to 100-\( \mu m \) diameter arterioles. Intermittent generation of endothelial NO provides a possible mechanism to enhance \( sGC \) activation by minimizing NO losses to the blood. The latter hypothesis is deemed plausible because both pulsatile changes in vascular networks and muscular contraction/relaxation have been shown to exhibit cyclic behavior with periods of \(< 1 \) s (21, 35, 37).

However, experimental validation of this transient mechanism will require real-time monitoring of NO concentration at resolutions on the order of milliseconds.

**APPENDIX**

**Dimensionless form of governing equation and boundary conditions.** The transient boundary value problem of Eqs. 1, 2, and 3 is expressed in dimensionless form as

\[
\frac{\partial \phi}{\partial \tau} = \frac{1}{y} \frac{\partial}{\partial y} \left[ y \frac{\partial \phi}{\partial y} \right] - \frac{R_3^2 R_{NO}(C)/D}{H_1} \frac{\partial^2 \phi}{\partial C^2} \quad (A1)
\]

\[
C(t, y = y_0) = 0 \quad \text{at} \quad y = y_0 = R/R_1 \quad (A2)
\]

\[
\frac{\partial C}{\partial y} \frac{\partial y}{\partial \tau} \bigg|_{y = y_1} = \frac{Q_1}{y_1} \quad \text{at} \quad y = y_1 = 1 \quad (A3)
\]

\[
\frac{\partial C}{\partial y} \frac{\partial y}{\partial \tau} \bigg|_{y = y_2} = \frac{Q_2}{y_2} \quad \text{at} \quad y = y_2 = R/R_1 \quad (A4)
\]

where \( y = r/R_1, \tau = D t / R_1^2, k^2 = k_1 R_3^2 / D, \) and \( H_1 = h R_1 / D. \)

\( Q_1 = S_{NO}/R_1 / D \) is the scaled production rate, \( R_3^2 R_{NO}(C)/D = k^2 C \) in pulmonary tissue (\( y_3 > y_i > 1 \)), and \( B_{NO}(C) = 0 \) in the EFZ (\( y_i \approx y = y_0 \)). The initial condition, at \( t = 0 \), corresponds to steady state with basal NO production levels, \( S_{NO,1}^0 \) and \( S_{NO,2}^0 \), at the endothelial membrane surfaces.

**Steady-state solution.** At steady state (SS) the time derivative in Eq. A1 vanishes and its solution is determined by direct integration of the resulting ordinary differential equation to obtain the following general solution

\[
C_{ss}(y) = C_{ss,1} \ln \left( y y_0 / (y_0 y_1) \right) \quad (A6)
\]

\[
C_{ss}(y) = f_1(y) C_{ss,1} - f_2(y) Q_{ss,1} \quad (A7)
\]

endothelial cell(s) (\( y_i \approx y \approx y_1 = 1 \))

\[
C_{ss}(y) = f_2(y) C_{ss,2} + g_2(y) \delta C_e \quad (A8)
\]

adventitial region (\( y_3 \approx y \approx y_2 \))

where \( Q_{ss,1} = S_{NO,1,ss} R_1 / D, \)

\( C_{ss,1} = C(t \rightarrow \infty, y = 1) \) and \( C_{ss,2} = \delta C / \eta_2 \) are known functions of the input parameters but are independent of the radial coordinate, \( f_1(y), f_2(y), g_1(y), \) and \( g_2(y) \) are known functions of both \( y \) and the input parameters, which satisfy Eqs. A2–A5, and are expressed in terms of Bessel functions.

**Transient solution.** We apply the method of separation of variables to obtain solutions to the governing equations, which are expressed in terms of exponential functions of time and Bessel functions in \( r (7, 15, 38) \). We then convert the resulting boundary value problem into Sturm-Liouville form, by expressing the solution as the sum of steady-state and transient parts. Application of Duhamel’s principle leads to analytical solution in terms of \( S_{NO,0}(t) \) (\( i = 1, 2 \)) (15).

The initial concentration distribution, \( C_{0,ss}(y) \) is assumed to be the steady state corresponding to \( S_{NO,1}(t = 0) = S_{NO,1}^0 \) (\( i = 1, 2 \)). Initially, we assume abrupt, simultaneous (step or pulse) changes in \( S_{NO,1}(t) \) from \( S_{NO,1}^0 \) to \( S_{NO,1}^{MAX} \) at both endothelial cell membranes (\( i = 1, 2 \)). Thus the scaled pro-

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duction rates, $Q(t) = S_{NO}(t)R_{i}/D$, have initial and final values $Q_0$ and $Q_{\text{Max}}$, evaluated at $S_{NO}$ and $S_{\text{Max}}$, respectively. We define the scaled change in total NO production as: $\Delta Q(t) = Q(t) - Q(t_0)$, where the maximum value, $\Delta Q_{\text{Max}}$, is computed at $Q_{\text{Max}}$. Thus the dimensionless changes in NO production rates are $q(t) = Q(t)/\Delta Q_{\text{Max}}$. Hence, the maximum values of $q(t)$ are $q_1^{\text{Max}} = \Delta Q_{\text{Max}}/Q_0$ and $q_2^{\text{Max}} = \Delta Q_{\text{Max}}/Q_{\text{Max}}$, where $q_1^{\text{Max}} + q_2^{\text{Max}} = 1$. Finally, we express the dimensionless concentration as $\Phi(y) = (C(t, y) - C_{0}(y))/\Delta Q_{\text{Max}}$.

For step changes in $q(t)$ the final, steady-state distribution, $\Phi_{\text{ss}}(y) = \Phi(y \rightarrow \infty)$, is determined within the three physiological regions of interest [the EFZ, endothelial cell(s), and the adventitial region] as a set of algebraic equations, which are linear in the inputs, $q_1^{\text{Max}}$ and $q_2^{\text{Max}}$. Employing superposition, we substitute $\Phi_{\text{ss}}(y) = \Phi_{\text{ss}}(y) + 0$ and $q_{\text{ss}}(y)$, substitute this sum into $Eqs. A1-A5$, and force $\Phi_{\text{ss}}(y)$ to satisfy $Eqs. A2-A5$. With either $q_{1}(t)$ or $q_{2}(t)$ set to zero (“turned off”), we determine the other solution for $\Phi_{\text{ss}}(y)$ by applying Duhamel’s principle (15). Finally, the transient concentration distribution is computed as $C(t, y) = C_{0}(y)/(\Phi(y, t_{\text{Max}}))$. For all three of the NO production scenarios considered here (i.e., step change, pulse change, and continuous (square wave) pulses), $Eqs. A1-A5$ can be integrated analytically. However, the final solutions are quite involved and are omitted for brevity.

REFERENCES


