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Nitric oxide from nitrite reduction by hemoglobin in the plasma and erythrocytes

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Abstract

Experimental evidence has shown that nitrite anion plays a key role in one of the proposed mechanisms for hypoxic vasodilation, in which the erythrocyte acts as a NO generator and deoxygenated hemoglobin in pre-capillary arterioles reduces nitrite to NO, which contributes to vascular smooth muscle relaxation. However, because of the complex reactions among nitrite, hemoglobin, and the NO that is formed, the amount of NO delivered by this mechanism under various conditions has not been quantified experimentally. Furthermore, paracrine NO is scavenged by cell-free hemoglobin, as shown by studies of diseases characterized by extensive hemolysis (e.g., sickle cell disease) and the administration of hemoglobin-based oxygen carriers. Taking into consideration the free access of cell-free hemoglobin to the vascular wall and its ability to act as a nitrite reductase, we have now examined the hypothesis that in hypoxia this cell-free hemoglobin could serve as an additional endocrine source of NO. In this study, we constructed a multicellular model to characterize the amount of NO delivered by the reaction of nitrite with both intraerythrocytic and cell-free hemoglobin, while intentionally neglecting all other possible sources of NO in the vasculature. We also examined the roles of hemoglobin molecules in each compartment as nitrite reductases and NO scavengers using the model. Our calculations show that: (1) ~0.04 pM NO from erythrocytes could reach the smooth muscle if free diffusion were the sole export mechanism; however, this value could rise to ~43 pM with a membrane-associated mechanism that facilitated NO release from erythrocytes; the results also strongly depend on the erythrocyte membrane permeability to NO; (2) despite the closer proximity of cell-free hemoglobin to the smooth muscle, cell-free hemoglobin reaction with nitrite generates approximately 0.02 pM of free NO that can reach the vascular wall, because of a strong self-capture effect. However, it is worth noting that this value is in the same range as erythrocytic hemoglobin-generated NO that is able to diffuse freely out of the cell, despite the tremendous difference in hemoglobin concentration in both cases (μM hemoglobin in plasma vs. mM in erythrocyte); (3) intraerythrocytic hemoglobin encapsulated by a NO-resistant membrane is the major source of NO from nitrite reduction, and cell-free hemoglobin is a significant scavenger of both paracrine and endocrine NO.

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Keywords

Computational model; Nitric oxide; Nitrite reduction; Intraerythrocytic hemoglobin; Cell-free hemoglobin

Nitric oxide (NO)¹ is a potent vasodilator that regulates vascular tone. It can be formed as the result of catalysis by nitric oxide synthase (NOS), which is found in the endothelium and the perivascular region of blood vessels. NO that diffuses into the vascular smooth muscle cells activates soluble guanylate cyclase (sGC), which further catalyzes the formation of 3',5'-cyclic guanosine monophosphate (cGMP) to produce vasodilation [1]. The paracrine NO derived from NOS activity has been regarded as the major source of the NO that induces vasodilation in the microvasculature [2].

In addition to the paracrine regulation of vascular tone by NO, endocrine signaling pathways involving the preservation and liberation of NO bioactivity by certain blood proteins have been identified [3,4]. Hemoglobin now appears to play a key role in the endocrine NO release from the lumen [5]. However, this molecule was initially regarded only as a potent NO scavenger because of its rapid reaction with NO in both oxygenated and deoxygenated states, with a kinetic rate limited only by diffusion. The stable end products of these reactions include methemoglobin (metHb), iron-nitrosyl-hemoglobin (HbNO), and nitrate. This NO scavenging effect causes vasoconstriction and impairs oxygen delivery. Encapsulation of hemoglobin inside erythrocytes reduces the scavenging effect because of the low permeability of the erythrocyte membrane to NO [6,7] and the erythrocyte-free zone inside the lumen that is formed when blood flows through arterioles [8]. However, cell-free hemoglobin, induced by hemolysis in sickle cell anemia or as a result of the administration of hemoglobin-based oxygen carriers, can enter the erythrocyte-free zone and extravasate into the interstitial space between the endothelium and smooth muscle. Experimental evidence has indicated that hypertension and vasoconstriction are associated with a significant presence of cell-free hemoglobin in blood [9,10]. Furthermore, computational modeling shows that cell-free hemoglobin can considerably reduce the bioavailability of enzymatic NO [11,12].

In addition to its role in decreasing NO bioactivity, hemoglobin has been proposed to sense the ambient O₂ change and to release NO under conditions of hypoxia, thereby causing an endocrine regulation of O₂ delivery. In one of the first hypotheses to account for endocrine NO signaling, it was suggested that the nitrosonium ion (NO⁺) can bind to the cysteine residue at position 93 in the hemoglobin β chain (β -93 cysteine) to form S-nitrosohemoglobin (SNOHb), in which the NO bioactivity is preserved. Under hypoxic conditions, hemoglobin undergoes allosteric changes that result in the transfer of NO to the thiols of the anion exchange protein present on the membrane, or to glutathione via transnitrosation. It is then further exported from the erythrocytes and induces vasodilation [3,13].

We have previously constructed a mathematical model to quantify the NO delivery by the intraerythrocytic SNOHb within the framework of the SNOHb hypothesis [14]. The model prediction showed the SNOHb released NO in the range 0.25–6 pM in the vascular wall and that this NO alone appeared to be insufficient to induce smooth muscle relaxation. Another competing hypothesis to account for endocrine NO signaling is related to nitrite reduction by deoxygenated hemoglobin. According to this hypothesis, nitrite is a stable reservoir that preserves NO bioactivity and is enzymatically reduced by hemoglobin to NO under hypoxic conditions, with the maximal nitrite reduction occurring around the P₅₀ of hemoglobin [15].

¹Abbreviations used: cGMP, 3',5'-cyclic guanosine monophosphate; NO, nitric oxide; NOS, nitric oxide synthase; NOS1, neuronal NOS; NOS2, inducible NOS; NOS3, endothelial NOS; SNOHb, S-nitrosohemoglobin; sGC, soluble guanylate cyclase.

The NO that is formed can reach the vascular smooth muscle either through free diffusion or in some protected forms that liberate NO close to the vascular wall. The chemistry involved in this reaction can be expressed as

Because of the complex chemical reactions involved in nitrite reduction and the difficulty that any change in NO from this source cannot be easily discriminated from NO released from other sources, there have been no direct experimental measurements *in vivo* of the amount of NO delivered to smooth muscle by this mechanism. Jeffers et al. [17], using a computational model, predicted a 0.08-pM NO presence in smooth muscle with free diffusion as the transport mechanism for NO after its production by the intraerythrocytic hemoglobin/nitrite reaction. However, NO delivery by potential facilitated mechanisms across the cell membrane and the cell-free hemoglobin/nitrite reaction have not yet been quantified.

Thus, hemoglobin apparently plays dual roles in regulating NO availability: It scavenges NO bioactivity to form metHb and HbNO, and it produces NO through nitrite reduction. Nitrite has been shown to be present in both erythrocytes and plasma in human blood [18]. Until now all studies of the reaction of hemoglobin with nitrite have been concentrating on erythrocyte-encapsulated hemoglobin. The possibility that cell-free hemoglobin can also react with nitrite, providing NO for vasodilation, has not been considered in detail. Because of cell-free hemoglobin is present in blood and can move freely into close proximity of the vascular wall, it is useful to determine whether it is capable of reducing nitrite to release sufficient NO for vasodilation, how the NO from this source compares to that from erythrocytes, and whether it can serve as an NO source, rather than merely a scavenger. In this study, we have constructed a computational model to analyze the NO release within the framework of this nitrite hypothesis, under physiological conditions and under conditions in which cell-free hemoglobin is present in high concentrations. We have also quantitatively analyzed the roles of hemoglobin as an NO scavenger and as a producer of NO through nitrite reduction.

Model formulation

Model geometry and assumptions

We have previously formulated a computational model that simulates NO release and transport in and around microvessels to predict the NO concentration in vascular smooth muscle when NO is liberated from the intraluminal SNOHb [14]. Here, we have modified the model to predict the smooth muscle NO exposure as a result of hemoglobin-mediated nitrite reduction. We considered a two-dimensional cross-section of an arteriole with its surrounding tissue, and a discrete distribution of erythrocytes in the lumen. The model consists of five layers as shown in Fig. 1: Layer 1 is the intraluminal layer containing plasma and discrete erythrocytes. Erythrocytes are modeled as circles. The erythrocyte membrane has been reported to have a low permeability (high resistance) to NO diffusion [19]. Thus, the cell is divided into two sub-regions: a thin membrane and the homogeneous intraerythrocytic hemoglobin. In Layer 1, NO can be formed as a result of the reaction occurring between nitrite and hemoglobin in erythrocytes and/or plasma. Layer 2 consists of the endothelium and associated interstitial space; Layer 3 is composed of smooth muscle cells containing sGC, which catalyzes the formation of cGMP; Layer 4 consists of non-perfused tissue containing nerve fibers and

parenchymal cells; and Layer 5 consists of tissue perfused by capillaries. The boundaries of each of the five layers are represented as circles.

In our model, we used 45% as the physiological hematocrit [20,21], which means that the area occupied by the erythrocytes in the lumen accounts for 45% of the total luminal cross-section. Our model also recognizes that the *in vivo* vessel hematocrit in arterioles may be significantly smaller than 45%, corresponding to the systemic hematocrit [22]. Thus, we also calculated the NO delivery with hematocrit values ranging from 15% to 45%. When a physiological erythrocyte-free layer was considered, erythrocyte positioning was limited by the rule that no part of an erythrocyte could be in the erythrocyte-free region.

To make the model tractable yet retain the most important features, we made the following assumptions: (1) The effect of convection of the blood flow is insignificant, as justified in [14,23]; (2) sources of NO other than nitrite/hemoglobin reaction are ignored, since in our model we are analyzing, one by one, the individual contributions of each biochemical pathway to the smooth muscle NO exposure; (3) the nitrite concentration does not change with time. Nitrite can be consumed during hypoxic vasodilation, but it can also be replenished by blood flow and its synthesis as a metabolite of NO bioactivity.

Governing equations and chemical reactions

The governing equation describing the diffusion of NO and the reactions of NO with the reactive species present in the vasculature is:

$$\frac{\partial C_{NO}}{\partial t} = D_{NO} \nabla^2 C_{NO} + R_{NO} \quad (1)$$

where C_{NO} is the NO concentration, D_{NO} is the diffusivity of NO, and R_{NO} is the sum of total production and consumption rates of NO. Here we considered the system at steady state

$\left(\frac{\partial C_{NO}}{\partial t} = 0\right)$. The governing equation was applied to all layers described above.

In the vasculature, NO can be consumed through reactions with a variety of species. In the perfused tissue layer (layer 5), NO is consumed by hemoglobin contained in erythrocytes flowing through the capillaries. NO also can react with oxygen. Also, we did not consider the NO production by the capillary endothelium, since we were focusing on the erythrocyte-induced hypoxic vasodilation. Moreover, we did not consider intracapillary NO production by the reaction between nitrite and hemoglobin in this layer because the nitrite reduction mediated by hemoglobin is maximal in precapillary arterioles [4]. In the non-perfused tissue layer (layer 4), there is no NO production, since the NOS activity was ignored when we focused on the NO vasodilation under hypoxic conditions. Also, NO is consumed in this layer through its reactions with oxygen. Here we also ignored the NO consumption by parenchymal tissue that occurs through an oxygen-dependent mechanism [24] because of the hypoxic conditions. In the smooth muscle layer (layer 3), NO is consumed through reactions with sGC, resulting in vasodilation. In the endothelium and the interstitial space (layer 2), NO is consumed through reactions with oxygen; the endothelial production of NO catalyzed by NOS3 was ignored here (see section Model assumptions). In the intraluminal layer (layer 1), the oxygen acts as a sink through its reactions with NO. When cell-free hemoglobin is considered, its reaction with nitrite present in the lumen produces NO; meanwhile, cell-free hemoglobin reacts with NO to form nitrate and methemoglobin or HbNO. Inside erythrocytes, NO is formed as nitrite is reduced by hemoglobin.

It is still unclear how the NO that is formed is transported out of erythrocytes. The likely mechanisms are [4]: (1) NO freely diffuses out of the erythrocyte; (2) the erythrocyte membrane-bound proteins provide a mechanism that facilitates NO transport without being rapidly scavenged by the intracellular hemoglobin; (3) another unknown species, not NO itself, is the immediate product of the nitrite and hemoglobin reaction. This unknown molecule is not reactive with hemoglobin and is converted to NO in the extracellular region. Case 1 was modeled as a free diffusion process during which NO also rapidly reacts with hemoglobin. We modeled Case 2 as a surface release determined by the availability of nitrite and intracellular hemoglobin, ignoring the intermediate processes. Because of the uncertainties about the reactivity of the intermediate products and the membrane protein-associated transport mechanism, we assumed that all of the formed NO molecules were able to leave the erythrocytes. Thus, the subset of these molecules that does not diffuse back into the previous erythrocyte or other erythrocytes could escape the hemoglobin scavenging. This facilitated release is unidirectional: The NO molecules that are released will encounter transport resistance when they diffuse back into the erythrocytes. This resistance can be specified by a high diffusivity in the thin membrane sublayer that is related to the membrane permeability [14, 19]. When NO diffuses back into the erythrocytes, it will rapidly react with the intraerythrocytic hemoglobin. Similarly, we modeled Case 3 as an instance of surface release but assigned an extremely low value to membrane permeability, effectively blocking the formed species from diffusing back into the erythrocytes and being scavenged by hemoglobin.

Layer 1 contains two sub-regions: the plasma and the erythrocytes. In each erythrocyte, NO is consumed through hemoglobin scavenging:

$$R_{\text{NO}} = -k_{\text{Hb}} C_{\text{Hb}} C_{\text{NO}} \quad (2)$$

where k_{Hb} is the kinetic reaction rate between hemoglobin and NO, C_{Hb} is the hemoglobin concentration, and C_{NO} is the NO concentration. A thin sub-layer along an erythrocyte was created to represent the erythrocyte membrane, which possesses an intrinsic resistance to NO diffusion. NO is formed from nitrite reduction and is then released outside the erythrocyte. When NO transport outside the cell occurs through free diffusion only, the NO production rate Q_{NO} inside the cell is:

$$Q_{\text{NO}} = k_{\text{nitrite}} C_{\text{Hb}} C_{\text{nitrite}} \quad (3)$$

where C_{nitrite} is the nitrite concentration and k_{nitrite} is the bimolecular reaction rate between nitrite and hemoglobin. When NO is exported through facilitated mechanisms, the release rate of this process is modeled as a surface reaction and expressed as a boundary flux, as discussed below. In the plasma sub-region, NO can freely diffuse in any direction. Also, the chemical reaction in the plasma sub-region is expressed as:

$$R_{\text{NO}} = -k_{\text{O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (4)$$

where k_{O_2} is the kinetic reaction rate between oxygen and NO and C_{O_2} is the oxygen concentration. When cell-free hemoglobin is present in this sub-region, its interactions with NO are described by Eqs. (2) and (3) for NO scavenging and nitrite reduction to NO, respectively.

In layer 2, the NO production catalyzed by NOS3 is ignored; NO is consumed through reaction with oxygen:

$$R_{\text{NO}} = -k_{\text{O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (5)$$

In layer 3, NO is consumed through reactions with sGC:

$$R_{\text{NO}} = -k_{\text{sGC}} C_{\text{NO}}^2 \quad (6)$$

where k_{sGC} is the kinetic reaction rate between sGC and NO.

In layer 4, the possible NO production by NOS1 is ignored; NO is consumed through the reaction with oxygen:

$$R_{\text{NO}} = -k_{\text{O}_2} C_{\text{O}_2} C_{\text{NO}}^2 \quad (7)$$

In layer 5, NO is consumed through reactions with oxygen and hemoglobin contained in erythrocytes flowing through the capillaries in the capillary-perfused region. Thus, the net reaction in this layer is:

$$R_{\text{NO}} = -k_{\text{O}_2} C_{\text{O}_2} C_{\text{NO}}^2 - k_{\text{cap}} C_{\text{NO}} \quad (8)$$

where k_{cap} is the effective reaction rate between NO and cellular hemoglobin in the capillaries.

Parameter values

In our calculations, the geometric information was similar to that in our previous study for SNOHb modeling: we chose 4 μm as the effective radius of an erythrocyte (r_1), which was modeled as a circle. The erythrocyte membrane is resistant to NO transport, and its thickness (r_{mem}) was chosen to be 0.0078 μm [25]. The radius of the pre-capillary arteriolar lumen (r_3) was chosen as 17 μm . We also simulated the effect of the erythrocyte-free zone adjacent to the vascular wall with a thickness of 2 μm , resulting in a region with a radius of 15 μm (r_2) that contains erythrocytes. We assumed that the thickness of the endothelium and that of the interstitial space was 0.5 μm , respectively. Thus, the total thickness of the endothelium and interstitial space layer was 1 μm and the radius (r_4) chosen for this layer was 18 μm . The radius (r_5) of the outer edge of the smooth muscle layer was 24 μm , with the consideration that the thickness of the smooth muscle was 6 μm [26]. The radius (r_6) of the non-perfused tissue layer was 30 μm , considering 6 μm as the thickness of the non-perfused tissue adjacent to the blood vessel. Finally, we chose 20 μm as the thickness of the perfused tissue, making the radius (r_7) of the entire region of our model 50 μm .

The diffusivity of NO inside erythrocytes is 880 $\mu\text{m}^2/\text{s}$ [19]. In all other regions, NO diffuses more rapidly, with a diffusivity of 3300 $\mu\text{m}^2/\text{s}$ [19,25]. The intracellular concentration of hemoglobin heme (C_{Hb}) was 20 mM. Under normal conditions, the cell-free hemoglobin concentration is low, approximately 1 μM . However, the cell-free hemoglobin concentration is significantly augmented, up to 20 μM , in hemolytic diseases or with the administration of

hemoglobin-based oxygen carriers [9]. Furthermore, the cell-free hemoglobin, unlike the much larger erythrocytes, can enter the erythrocyte-free zone or extravasate into the interstitial space between the endothelium and smooth muscle. We have also taken these effects into account in our model. NO can react with hemoglobin to form relatively stable metabolites, and the reaction rate (k_{Hb}) between hemoglobin and NO has been reported in the range from 12 to 90 $\mu\text{M}^{-1} \text{s}^{-1}$ [27-30]. Here we chose 18 $\mu\text{M}^{-1} \text{s}^{-1}$ as the kinetic rate [29], but also examined the effect of other possible rates on the NO delivery to smooth muscle (see section Results). The concentration of sGC (C_{sGC}) used was 0.1 μM , and the reaction rate between sGC and NO (k_{sGC}) was 0.05 $\mu\text{M}^{-1} \text{s}^{-1}$ [25]. The effective reaction rate between NO and the capillary hemoglobin (k_{cap}) was 12.4 s^{-1} , as justified in [31]. The reaction rate between oxygen and NO was $9.6 \times 10^{-6} \mu\text{M}^{-2} \text{s}^{-1}$ [31]. The ambient oxygen concentration was 52 μM (see below) as a constant. Although there is supposed to be a radial gradient of oxygen concentration, our calculations using a fixed nitrite reduction rate, but different values (0, 52, and 100 μM) as constant oxygen concentrations in each simulation, showed that there was little influence of O_2 as a reactant on NO distribution (data not shown).

The release rate of NO depends on the concentrations of hemoglobin and nitrite and their reaction kinetics. The intraerythrocytic nitrite concentration was reported to be 288 nM by Dejam et al. [18]. The reported nitrite concentration in plasma was 121 nM [18], although a much wider range of values has been reported because of methodological inconsistencies, as reviewed in [32]. It has been suggested that nitrite reduction rate is a function of the oxygenation of hemoglobin because of the involvement of both the R-state and T-state of the hemoglobin tetramer. The maximal rate occurs when hemoglobin is 50% saturated with oxygen [15,33]. In our model, we used $4.4 \times 10^{-6} \mu\text{M}^{-1} \text{s}^{-1}$ (k_{nitrite}) as the apparent reaction rate, which corresponds to a PO_2 of ~30 mm Hg (~52 μM) in the pre-capillary arteriolar region [15,33]. Thus, the volumetric NO production rate Q_{NO} was $2.53 \times 10^{-2} \mu\text{M/s}$ (calculated from Eq. (3)).

As discussed earlier, the NO molecules that are formed could be transferred to membrane-associated proteins, or to an intermediate product that is more stable, then further exported out of the cell. Thus, the NO group can avoid being scavenged through rapid rebinding to the hemoglobin iron. In our model, we represented this series of biochemical reactions using an apparent surface release reaction. The total amount of the released NO is the product of the volume of erythrocyte (V) and the NO production rate (Q_{NO} , determined above). Thus, the surface release rate of NO from the erythrocyte membrane per unit time per unit area was:

$S_{\text{NO}} = \frac{Q_{\text{NO}} \cdot V}{A}$, where A is the surface area of the erythrocyte. Both V and A are determined by the radius of the erythrocyte. Given the values of Q_{NO} and the radius of erythrocyte in Table 1, S_{NO} was $3.4 \times 10^{-17} \mu\text{mol} \mu\text{m}^{-2} \text{s}^{-1}$. The production of NO by the erythrocyte-NO was incorporated as surface NO release into the boundary conditions at the interface between the erythrocyte membrane and the plasma (see section Boundary conditions). All the parameter values, including the size (radius) information of each layer that is based on experimental data for arterioles, are listed in Table 1.

Boundary conditions

All boundaries between the regions described in the ‘Model geometry’ section had continuous NO concentration distributions, except for the interface between the erythrocyte membrane and the plasma, where a finite transport resistance was introduced. Here we assumed that the solubility of NO in each region in the model is the same. At the outer interface of the whole region, we assumed a no-flux condition. The boundary conditions were:

1. At the outer boundary of layer 5:

$$\frac{\partial C_{\text{NO}}}{\partial r} = 0 \quad (9)$$

2. At the interface between the plasma and the erythrocyte, the NO release from the erythrocyte was incorporated as the surface boundary condition:

$$S_{\text{NO}} = D_{\text{NO,mem}} \frac{\partial C_{\text{NO,mem}}}{\partial r} - D_{\text{NO,plasma}} \frac{\partial C_{\text{NO,plasma}}}{\partial r} \quad (10)$$

where S_{NO} is the NO release from the erythrocyte surface through the membrane protein-associated mechanism, $D_{\text{NO,mem}}$ is the diffusivity of NO in the erythrocyte membrane, $D_{\text{NO,plasma}}$ is the diffusivity of NO in the plasma, $C_{\text{NO,mem}}$ is the NO concentration in the thin erythrocyte membrane layer, and $C_{\text{NO,plasma}}$ is the NO concentration in plasma. In the membrane of each erythrocyte, the apparent diffusivity of NO was determined by the membrane permeability (P_{mem}) and the membrane thickness (r_{mem}) [19]:

$$D_{\text{NO,mem}} = P_{\text{mem}} \cdot r_{\text{mem}} \quad (11)$$

The membrane permeability (P_{mem}) was chosen to be 450 $\mu\text{m/s}$ [19,25], although other values for this parameter have been reported [7,34]. The membrane thickness (r_{mem}) was 0.0078 μm [19]. Thus, the apparent diffusivity of NO inside the erythrocyte membrane was 3.51 $\mu\text{m}^2/\text{s}$. In our numerical calculations, we used a larger r_{mem} (0.078 μm , a value still much less than the erythrocyte size) and therefore a higher $D_{\text{NO,mem}}$ (35.1 $\mu\text{m}^2/\text{s}$), in order to make our numerical solution more efficient. This approximation has been justified in our previous study [14].

Numerical method

The governing equation (Eq. (1)) coupled with the appropriate boundary conditions was solved numerically using the FlexPDE software package (PDESolutions, Antioch, CA). The code was implemented on a 3-GHz processor with 2-GB synchronous dynamic random access memory (SDRAM).

Results

NO delivery by intraerythrocytic nitrite reduction

As discussed in section Model formulation, three mechanisms of NO delivery by nitrite reduction from erythrocytes are likely: (1) free diffusion; (2) a facilitated mechanism across the erythrocytic membrane; and (3) the formation of a non-reactive intermediate that liberates NO once the vessel wall is reached. We calculated the NO distribution around an arteriole with 45% hematocrit when these three mechanisms were considered. Fig. 2a and b shows that when nitrite reduction occurred uniformly inside erythrocytes and the formed NO freely diffused out of the cells, the NO concentration level inside the lumen was sub-picomolar, and the concentration of NO present in smooth muscle was about 0.04 pM. If the NO bioactivity were protected after it was formed and was then released through a facilitated mechanism across the cell membrane, as postulated in certain hypotheses [4], the NO presence in smooth muscle would be about 43 pM (Fig. 2c and d), a value about three orders of magnitude higher than that shown in Fig. 2b.

If the exported NO bioactivity was in the form of a yet-unknown species that is inactive, diffuses out to the vascular wall and liberates NO molecules there, the NO delivered to smooth muscle by such a mechanism would be significantly increased. For such a scenario, we estimated the NO level in smooth muscle to be about 260 pM (Fig. 2e and f). It should be noted that the diffusivity and reaction kinetics of this assumed and currently unknown species in lumen and perivascular region are not yet clear; the 260 pM calculated here should be regarded as an estimate until the detailed characteristics of this intermediate species, if it exists, are experimentally identified.

In our model, we used 450 $\mu\text{m/s}$ as the membrane permeability (see Table 1). However, it should be noted that the lipid bilayer of the erythrocyte membrane was initially thought to be highly permeable to NO [2]. Later experiments and modeling [19,25] showed that the cell membrane poses an intrinsic barrier to NO transport. Nevertheless, other studies [34,35] suggested that the membrane permeability may be higher because the slow NO binding rate to intraerythrocytic hemoglobin observed in Refs. [19,25] might be due to other hematocrit-dependent mechanisms (e.g. extracellular diffusion). Thus, we also calculated the NO concentration in smooth muscle under the same conditions as in Fig. 2c and d except for using a high value of P_{mem} (45,000 $\mu\text{m/s}$), a value reported by Liu et al. [34]. The concentration we obtained with this higher P_{mem} was 2.0 pM in smooth muscle, which is considerably lower than the 43 pM with low P_{mem} . The nature of the membrane resistance to the uncharged NO requires further investigation to gain a more complete understanding of endocrine NO signaling.

Our calculations were performed for a regular distribution of erythrocytes (Fig. 2). Other random types of distribution of the cells in the lumen, with the constraint that there was no overlap between them, had no significant effect on the results, as shown in our previous studies on NO release and transport from other intraerythrocytic sources [14]. Furthermore, because of the relatively large range of reported values of NO and hemoglobin reaction rate (k_{Hb} from 12 to 90 $\mu\text{M}^{-1} \text{s}^{-1}$; [27-30]), we also simulated the NO delivery with a different k_{Hb} . Our calculations showed that, with the NO-hemoglobin reaction rate of 90 $\mu\text{M}^{-1} \text{s}^{-1}$, the NO delivered through the mechanism in Fig. 2a and b dropped nearly fivefold, but NO delivery through the mechanism in Fig. 2c and d decreased just slightly (<1%), and no difference exists in the formation of a non-reactive intermediate that does not liberate NO until reaching the vessel wall (the mechanism in Fig. 2e and f).

NO delivery by intraerythrocytic nitrite reduction as a function of hematocrit

The influence of the hematocrit on the delivery of NO to the smooth muscle was also tested by varying the hematocrit value. Our calculations showed that the NO concentration was not linearly proportional to the change in hematocrit. When the hematocrit was dropped from 45% to 30%, the NO concentration in smooth muscle was slightly reduced to 41 pM, and this value became about 31 pM with a 15% hematocrit. This nonlinear relationship presumably reflects the fact that the released NO can be scavenged not only by the hemoglobin in the erythrocytes from which the NO molecules were released, but also by hemoglobin in other erythrocytes. Our result suggests that the change in hematocrit under physiological conditions would not produce a relatively large variation in exposure of the smooth muscle to NO.

NO availability when nitrite reduction by cell-free hemoglobin is the sole source of NO

We also simulated the availability of NO produced by the reaction between nitrite and hemoglobin in plasma. We considered the case in which 20 μM cell-free hemoglobin was uniformly distributed within the blood plasma, including the cell-free zone adjacent to the vascular wall. The reaction product from nitrite reduction in our simulations was free NO, because it is unlikely that the nitrogen oxide species formed in the extracellular space are

protected by membrane-associated proteins (such as band 3 protein). Our calculations (Fig. 3) showed that the NO concentration in the lumen and arteriolar wall is around 0.02 pM, a value close to that for NO delivered by an intraerythrocytic hemoglobin/nitrite source under conditions of free diffusion (Fig. 2a and b), but far less than that expected if the membrane mechanisms facilitated the release of NO from the intracellular sources (Fig. 2c and d). This 0.02 pM concentration of NO seems negligible in terms of vasoactivity, when compared with the amount of NO required for vasodilation (discussed below).

In our calculations in Fig. 3 we used a high cell-free hemoglobin concentration (20 μ M), which corresponds to the level in certain hemolytic diseases. Under normal physiological conditions, the cell-free hemoglobin level is significantly lower. Simulations below will show that the amount of NO derived from this source alone is even lower under normal physiological conditions.

NO delivery as a function of nitrite and hemoglobin concentrations

We have calculated the amount of NO that reaches the vascular wall after nitrite reduction by intracellular and extracellular hemoglobin, respectively. Previous studies have shown that cell-free hemoglobin causes vasoconstriction because of the scavenging of paracrine NO [9]. Here we asked whether the presence of a higher amount of cell-free hemoglobin could produce more endocrine NO to offset the loss of NO production catalyzed by NOS. Fig. 4a shows the levels of NO delivered to vascular smooth muscle for different amounts of cell-free hemoglobin. When the nitrite concentration was fixed at 0.121 μ M, the availability of NO from this source increased with higher cell-free hemoglobin concentration but reached a plateau at approximately 80 μ M cell-free hemoglobin, presumably because of the balance between NO production and scavenging by cell-free hemoglobin. The highest possible contribution of NO to the vascular wall from the cell-free hemoglobin/nitrite reaction was below 0.03 pM (Fig. 3a), when the nitrite level was 0.121 μ M. This result indicates that the ability of cell-free hemoglobin to generate net NO by reducing nitrite is negligible. Nevertheless, the NO delivery from this source is nearly proportional to the available nitrite in the plasma region. Fig. 4b shows that with a fixed concentration of cell-free hemoglobin (20 μ M), the NO level at the vascular wall increases linearly with higher amounts of nitrite available in the plasma.

We also calculated the NO delivery as a function of nitrite for the case in which intraerythrocytic hemoglobin reacted with nitrite to produce NO that was then transported by means of a facilitated mechanism. A linear relationship with the nitrite concentration was found for this intracellular source (data not shown). Because the intraerythrocytic hemoglobin concentration is a relatively stable value at around 20 mM, we did not consider the effect of its variation on NO delivery.

Extravasation of cell-free hemoglobin into the interstitial space

Cell-free hemoglobin, unlike that encapsulated in erythrocytes, can extravasate into the interstitial spaces adjacent to vascular smooth muscle. The presence of cell-free hemoglobin in the interstitial space is thought to further reduce the enzymatic NO availability and thus induce vasoconstriction [11,12], although the mechanism behind the vasoconstriction induced by this cell-free hemoglobin is under debate [36]. We compared the NO levels in the smooth muscle resulting from the cell-free hemoglobin/nitrite interaction alone, assuming that extravasation did or did not occur. Fig. 5 shows that the presence of 20 μ M hemoglobin in the interstitial space did not significantly increase the NO delivery through nitrite reduction by cell-free hemoglobin, despite its closer location to the vascular wall. Also, based on the results from Fig. 4a, a higher concentration of cell-free hemoglobin that extravasated into the interstitial space would not significantly increase the NO delivery because of the interplay of the nitrite reductase activity and NO scavenging of hemoglobin.

Endocrine NO distribution when intraerythrocytic and cell-free hemoglobin reducing nitrite are considered

We also considered the NO distribution when both intracellular and extracellular sources were included in our model. Fig. 6a shows that without a membrane-facilitated mechanism, the total NO in the vascular wall resulting from nitrite reduction by both intracellular and extracellular hemoglobin was 0.037 pM for 1 μ M cell-free hemoglobin. This value dropped to 0.027 pM when 30 μ M cell-free hemoglobin was present. It should be noted that the intracellular source alone could deliver about 0.04 pM (Fig. 2b), and 30 μ M cell-free hemoglobin with nitrite could deliver about 0.02 pM NO (Fig. 4a) to smooth muscle. This result is very important because this is the first theoretical estimation of the separate contributions of erythrocyte-enclosed and cell-free hemoglobin to nitrite reduction to NO. It strongly indicates that a membrane-associated mechanism to facilitate NO export out of erythrocytes is necessary for NO signaling through nitrite reduction. Fig. 6b shows that with a membrane-facilitated mechanism operating across the erythrocyte membrane, the concentration of NO was about 37 pM at the vascular wall when 1 μ M hemoglobin was present in the plasma; however, this value was reduced to about 5 pM with a higher cell-free presence (30 μ M). The intracellular source could deliver about 40 pM (Fig. 2d) through a facilitated mechanism, and 30 μ M cell-free hemoglobin with nitrite could deliver about 0.02 pM NO (Fig. 4a) to smooth muscle. Since both intracellular and extracellular nitrite reduction reactions were considered here, the final NO exposure at the vascular wall was not just a sum of the NO from these two sources but a combination of the net effect of NO production and scavenging. A higher concentration of cell-free hemoglobin resulted in lower NO delivery from intraluminal NO reduction. Thus, our calculations indicate that the major role of cell-free hemoglobin in endocrine NO signaling is still as a scavenger, by analogy to its role in paracrine NO signaling.

Discussion

In the present study, we have constructed a multicellular computational model to quantify NO delivery to the vascular wall after intraluminal nitrite reduction by hemoglobin under hypoxic conditions. We simulated the NO transport that occurs after nitrite reduction in erythrocytes, considering three distinct transport mechanisms: free diffusion, a membrane-associated mechanism, and a mechanism involving an intermediate product that only liberated NO as it reached the vascular wall. We also calculated the NO production resulting from the reaction between cell-free hemoglobin and nitrite and the subsequent NO transport. We further tested the scavenging effect of cell-free hemoglobin on endocrine NO and identified the importance of a protected mechanism to transport the formed NO across the cell membrane.

Comparison with previous studies

As discussed earlier, there have been no direct experimental measurements *in vivo* of the amount of NO delivered through nitrite reduction because of the complex chemistry involved in this mechanism. Other modeling studies by Jeffers et al. [17] have reported that the NO concentration in smooth muscle is 0.08 pM after intraerythrocytic nitrite is reduced to NO, which is then transported through free diffusion. Our calculation for the intraerythrocytic sources, which predicted a level of ~0.04 pM NO (Fig. 2a and b), is in good agreement with their value. The slight discrepancy between our model and that of Jeffers et al. likely reflects the different handling of the NO sinks in the capillary-perfused region. Both predictions point to a sub-picomolar NO level if there is no protection mechanism for the NO molecules formed. However, both models will need to be tested experimentally once detection approaches become available that can measure endocrine NO in the vessel lumen.

Our model also predicts that NO is present in smooth muscle at 43 pM (Fig. 2c and d) if the formed NO can be released from erythrocytes through a facilitated mechanism. This value

could be increased to around 260 pM (Fig. 2e and f) in an extreme case if an intermediate of the reactions exists, which would carry the NO and liberate it before reaching the vascular wall. In [17], the authors also pointed out that 8 pM NO could reach the smooth muscle from erythrocytes, provided that the formed NO or intermediate species had a longer lifetime (1 ms). Our values for the case in which protected forms of NO transport existed were significantly higher than 8 pM. Furthermore, these values were higher than the levels of SNOHb-released NO predicted by a computational model within the framework of the SNOHb hypothesis, which predicts 0.25–6 pM NO in smooth muscle, depending on the intracellular concentration of SNOHb [14]. However, it should be noted that such protected transport mechanisms have not yet been experimentally identified.

The role of cell-free hemoglobin in NO bioavailability

Experimental evidence [9,10] and theoretical modeling [11,12,37] have established that the presence of cell-free hemoglobin in blood significantly reduces the enzymatic NO bioavailability. However, the possibility of NO release in the reaction of cell-free hemoglobin with nitrite brought us to ask whether cell-free hemoglobin could also serve as a NO producer, rather than scavenger, under hypoxic conditions in which the enzymatic NO level is greatly attenuated. According to results from our simulations, this scenario is unlikely: Fig. 3 shows that under typical hemolytic conditions in which 20 μ M cell-free hemoglobin is present in the lumen, only $\sim$ 0.02 pM NO reaches smooth muscle. This result suggests that although the nitrite reduction by cell-free hemoglobin can occur close to the vascular wall, the NO delivery is still negligible. Under such conditions, the NO production rate from this source is low ($\sim$ 11 pM/s as the K_{nitrite} is $4.4 \times 10^{-6} \mu\text{M}^{-1} \text{s}^{-1}$) and thus presumably cannot significantly contribute to the steady-state NO concentration profile. Also, the self-capture effect of the newly formed NO by hemoglobin itself further limits the NO availability from this source. A higher level of cell-free hemoglobin can enhance the NO release from this source, but it also increases the scavenging rate of NO by hemoglobin, leading to a plateau in the NO concentration profile (Fig. 4a). Thus, a more concentrated cell-free hemoglobin presence does not result in more net NO from this source; instead, it strengthens the scavenging effect of NO from other endocrine (Fig. 6) and paracrine sources.

The limited degree of NO delivery mediated by cell-free hemoglobin/nitrite reactions was not increased by the extravasation of the free hemoglobin into the interstitial space, which is proximal to the smooth muscle. It has been speculated that a closer location of the intraluminal NO sources to the vascular wall could significantly facilitate NO delivery to smooth muscle [38]. However, our calculations (Fig. 5) show that, without any protective mechanisms, few NO molecules can freely diffuse to smooth muscle, even when the nitrite reduction mediated by cell-free hemoglobin occurs just next to the target. Thus, it is not the location of the reaction that matters for NO delivery resulting from nitrite reduction; instead, other mechanisms involving the erythrocyte membrane or intermediate species formed during the reactions make endocrine NO signaling possible.

The importance of the protected transport of NO

It is worth noting that if the NO formed through nitrite reduction is transported only through free diffusion, the NO derived from intraerythrocytic sources ($\sim$ 0.04 pM; Fig. 2a and b) is comparable to that derived from nitrite reaction with cell-free hemoglobin ($\sim$ 0.02 pM; Fig. 3), which is widely regarded as a potent scavenger. In addition, both appear to be negligible in terms of their effect on vasodilation, since they are far below the reported EC_{50} of NO for sGC activation (discussed below). This situation raises the question of how hemoglobin and nitrite in erythrocytes can deliver significant amounts of NO to the vascular wall to produce the hypoxic vasodilation that is observed experimentally [4]. A membrane-associated mechanism (rather than free diffusion) that protects NO bioactivity and facilitates its export out of

erythrocytes is one possible mechanism; the formation of an inactive (or significantly less active) intermediate species that releases NO only when it is transported to the vascular wall is another likely mechanism, as shown in the simulations in Fig. 2. After NO leaves the erythrocytes, its diffusion back into the cells encounters the resistance posed by the erythrocyte membrane, which in conjunction with the protected export mechanism facilitates NO delivery from the intraerythrocytic source to the vascular wall. Our simulations show that this situation can enhance the NO delivery by approximately three orders of magnitude or even more, when compared to the effect of free diffusion (Figs. 1 and 2). Furthermore, when NO in the lumen diffuses back into erythrocytes, it may encounter resistance because of the low membrane permeability (discussed in Results). Taken together, the erythrocyte membrane appears to play an important role in differentiating between the nitrite reduction by intraerythrocytic and cell-free hemoglobin and is essential to endocrine NO signaling.

Effect of NO from nitrite reduction in capillary

We have considered the NO concentration profile around an arteriole when the nitrite reduction by hemoglobin in the arteriolar lumen is the sole NO source. In our model, Layer 5 represents the tissue perfused by capillaries. Because of the existence of nitrite and erythrocytes in this layer, the nitrite reduction in capillaries could alter the radial NO gradients. To test this possibility, we modified our model by adding the NO generation from the capillary-perfused region due to nitrite reduction. The bimolecular reaction rate between nitrite and hemoglobin was $4.4 \times 10^{-6} \mu\text{M}^{-1} \text{s}^{-1}$, though it could be lower because of the different oxygen conditions in capillaries than in arterioles [15,33]. Thus, the volumetric NO production from an erythrocyte was still $2.53 \times 10^{-2} \mu\text{M/s}$ (Eq. (3)). The NO generation from nitrite reduction in capillaries with complex geometry in Layer 5 was simplified by considering a homogenous NO production. It has been shown that the fractional volume of capillaries in hamster retractor muscle is 0.0146 [23,39]. Given a 30% capillary hematocrit, the effective volumetric NO production from the capillary-perfused region was calculated to be 0.11 nM/s (multiplication of NO generation rate from a single erythrocyte, capillary hematocrit, and the fractional volume of capillaries). Fig. 7 shows that, when free diffusion was the transport mechanism, the NO from nitrite reduction in capillaries did not significantly alter the NO concentration around an arteriole and only accounted for a small portion (~0.5%) of the NO in smooth muscle. The NO contribution from this source, when the facilitated transport mechanism was present, is expected to be small in contrast to that from arterioles because of the 0.5% ratio above (presumably due to the small fractional volume). However, a detailed model with the complex capillary network geometry is needed in future studies to answer this question quantitatively.

Is erythrocyte-produced NO sufficient to induce vasodilation?

We have predicted a 40–260 pM concentration of NO in smooth muscle, assuming that the protected forms of the nitrogen oxide species derived from nitrite reduction actually exist. Whether this concentration range is sufficient to induce vasodilation depends on the EC_{50} of NO for sGC activation, which is responsible for smooth muscle relaxation. EC_{50} was initially reported to be as high as 250 nM *in vitro* [40]. Recent studies have pointed to the amount of NO required to activate sGC to half-maximal activity as being on the nanomolar level [41, 42] or even sub-nanomolar [43]. Our predicted NO values obtained for the protected transport form were close to the sub-nanomolar EC_{50} . Likely a nanomolar level of NO produced by the endothelium would be required to match the nitrite reduction activity, depending on further understanding of the interaction of NO and sGC. Moreover, it has been shown that, under therapeutic conditions, the application of a higher concentration of nitrite can increase the blood flow by 22% at 2.5 μM and by 175% at 200 μM , likely through the NO-sGC-cGMP pathway [44]. Our simulations show that 0.4 nM (if a facilitated membrane mechanism exists) or 2.3 nM (if a non-reactive intermediate forms that does not liberate NO until reaching the vessel wall) NO would be delivered to smooth muscle with 2.5 μM nitrite; this amount increases to

30 or 182 nM (corresponding to the two cases above, respectively) if 200 μ M nitrite is applied. The NO delivery by nitrite reduction under such therapeutic conditions appears to be sufficient to induce vasodilation. However, a clear understanding of the EC₅₀ of NO for sGC is required to determine the actual potency of NO derived from nitrite reduction by intraerythrocytic and cell-free hemoglobin.

Another question remains to be addressed: There is a significant discrepancy [45,46] between the predicted values for the enzymatic NO concentration and the perivascular values measured in vivo with NO microelectrodes, which are orders of magnitude higher; these values have been reported in the range of 200–600 nM under controlled conditions [47–49]. Such values point to the existence of other pathways that lead to NO release. Apparently, the NO released from the nitrite reservoirs under physiological conditions does not account for this discrepancy.

In conclusion, we have used a mathematical model to predict the level of NO delivered to arteriolar smooth muscle through nitrite reduction by intraerythrocytic hemoglobin as a result of the operation of three different transport mechanisms across the cell membrane: (1) NO freely diffusing out of the erythrocyte, (2) the erythrocyte membrane-bound proteins providing a mechanism that facilitates NO transport without it being rapidly scavenged by intracellular hemoglobin; (3) an unknown non-reactive species, the immediate product of the nitrite and hemoglobin reaction, stimulating the release of NO as this species reaches vascular wall. Our calculations predicted concentration of approximately 0.04 pM, 43 pM, and 260 pM in smooth muscle, respectively, for these three mechanisms. We also predicted that despite the closer proximity of cell-free (vs. erythrocyte) hemoglobin to smooth muscle, only approximately 0.02 pM NO can reach the vascular wall from the nitrite source because of a strong capture effect by hemoglobin itself. Our calculations show that cell-free hemoglobin is a significant scavenger of both paracrine and endocrine NO. The protected transport of the nitrite reduction from inside the erythrocyte across cell membrane, coupled with the intrinsic resistance of the membrane to NO transport from extracellular space to intracellular region, must play an important role in endocrine NO signaling from nitrite reservoirs.

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Fig. 1. Model schematic. The model consists of five layers: (1) the intraluminal layer containing discrete erythrocytes and plasma; (2) endothelium and the interstitial space; (3) smooth muscle cells; (4) non-perfused tissue containing nerve fibers and parenchymal cells; and (5) tissue perfused by capillaries. NO from nitrite reduction by hemoglobin is the sole source of NO in the model.

Fig. 2.

NO concentration profiles when nitrite reduction by intraerythrocytic hemoglobin is the sole source of NO. The contour plots (a, c, and e) represent the cross-sections of an arteriole that contains erythrocytes and its surrounding tissue. The elevation plots (b, d, and f) show the NO concentration from the center of the lumen to the perivascular region along a path. The starting and ending points of the path are represented as 1 and 2, respectively, in the inset. The small circles in the inset represent erythrocytes. NO delivery by nitrite reductions is calculated under three conditions: (1) NO formation by nitrite reduction occurs uniformly inside erythrocytes and the subsequent transport of NO occurs through free diffusion (a and b); (2) formed NO is transported out of the cell membrane through a facilitated mechanism (c and d); (3) the NO

bioactivity is preserved as an intermediate species, which does not liberate NO until it reaches the vascular wall (e and f). The erythrocyte membrane permeability was 0.001 $\mu\text{m/s}$ in (e) and (f). All other parameters are listed in Table 1. Hematocrit was 45%. The NO concentration inside each erythrocyte was nearly zero.

Fig. 3.

NO concentration profile when nitrite reduction by cell-free hemoglobin is the sole source of NO. The concentration of cell-free hemoglobin was 20 μM . The reduction of nitrite by intracellular hemoglobin was not considered. The erythrocyte distribution and other simulation conditions were the same as in Fig. 2. The contour plot (a) represents the cross-sections of an arteriole that contain erythrocytes and its surrounding tissue. The elevation plot (b) shows the NO concentration from the center of the lumen to the perivascular region along a path. The starting and ending points of the path are presented as 1 and 2, respectively, in the inset. The circles in the inset represent erythrocytes, which are sinks for NO. NO is produced by the reaction between cell-free hemoglobin and extracellular nitrite.

Fig. 4.

NO delivered to smooth muscle through nitrite reduction by cell-free hemoglobin, as a function of the (a) cell-free hemoglobin concentration or (b) extracellular nitrite concentration. All other NO production sources, including nitrite reduction by intracellular hemoglobin, were not considered in the simulation. In (a), the concentration of extracellular nitrite was $0.121 \mu\text{M}$ and that of cell-free hemoglobin was varied from 1 to $100 \mu\text{M}$. In (b), the concentration of cell-free hemoglobin was $20 \mu\text{M}$ and that of nitrite was varied from 0.121 to $30 \mu\text{M}$. The erythrocyte distribution and other simulation conditions were the same as shown in Fig. 3.

Fig. 5.

The effect of extravasation of cell-free hemoglobin into the interstitial space on the endocrine signaling of NO reduced by nitrite. In addition, the presence of 20 μM cell-free hemoglobin in the lumen was also included in the simulation in the case of extravasation. The concentration of the cell-free hemoglobin in the interstitial space was the same as that in the lumen (20 μM).

Fig. 6.

NO concentration profiles when NO production from nitrite reduction by both intracellular and extracellular hemoglobin is considered. (a) NO release from erythrocytes occurs through the facilitated mechanism, as discussed in section Model formulation; (b) NO formation in erythrocytes occurs uniformly inside the cells, and NO transport out of the cells occurs through free diffusion.

Fig. 7.

Contribution of nitrite reduction in capillaries to the NO concentration around an arteriole. The homogeneous NO production rate from the capillary-perfused region was 0.11 nM/s. All other simulation conditions were the same as those in Fig. 2a and b. The NO concentration curves, when the nitrite reduction in capillaries was considered or not, nearly overlapped, indicating that NO from this source did not significantly alter the NO concentration around an arteriole.

Table 1

Model parameters

Parameters	Value	Unit	Reference
Radius			
Erythrocyte (r_1)	4	μm	[19,25]
Erythrocyte-rich layer (r_2)	15	μm	Text
Erythrocyte-free layer (r_3)	17	μm	Text
Endothelium and interstitial space (r_4)	18	μm	Text
Smooth muscle layer (r_5)	24	μm	Text
Non-perfused tissue (r_6)	30	μm	Text
Perfused tissue (r_7)	50	μm	Text
Erythrocyte membrane thickness (r_{mem})	0.0078	μm	[19,25]
Erythrocyte membrane permeability (P_{mem})	450	$\mu\text{m/s}$	[19,25]
NO diffusion coefficient in membr (D_{mem})	3.51	$\mu\text{m}^2/\text{s}$	Text
Extracellular NO diffusion coefficient (D_{ext})	3300	$\mu\text{m}^2/\text{s}$	[19]
Intracellular NO diffusion coefficient (D_{int})	880	$\mu\text{m}^2/\text{s}$	[25]
Hemoglobin–NO reaction rate (k_{Hb})	18	$\mu\text{M}^{-1} \text{s}^{-1}$	[29]
Plasma nitrite concentration	121	nM	[18]
Intraerythrocytic nitrite concentration	288	nM	[18]
Intracellular hemoglobin concentration	20	mM	Text
Cell-free hemoglobin concentration	0–20	μM	[9]; text
Reaction rate with sGC (k_{sGC})	0.05	$\mu\text{M}^{-1} \text{s}^{-1}$	[25]
Oxygen concentration (C_{O_2})	52	μM	Text
Reaction rate with O_2 (k_{O_2})	9.6×10^{-6}	$\mu\text{M}^{-2} \text{s}^{-1}$	[31]
NO consumption rate by perfused tissue (K_{cap})	12.4	s^{-1}	[31]; Text
Nitrite and hemoglobin reaction rate (K_{nitrite})	4.4×10^{-6}	$\mu\text{M}^{-1} \text{s}^{-1}$	[15]
NO surface release rate (S_{NO})	3.4×10^{-17}	$\mu\text{mol} \mu\text{m}^{-2} \text{s}^{-1}$	Text

The radius of each layer, except r_1 , represents the distance from the center of the lumen to the outer boundary of the layer.