

Review

Nitric Oxide in the Vasculature: Where Does It Come From and Where Does It Go? A Quantitative Perspective

KEJING CHEN,¹ ROLAND N. PITTMAN,² and ALEKSANDER S. POPEL¹

ABSTRACT

Nitric oxide (NO) affects two key aspects of O₂ supply and demand: It regulates vascular tone and blood flow by activating soluble guanylate cyclase (sGC) in the vascular smooth muscle, and it controls mitochondrial O₂ consumption by inhibiting cytochrome *c* oxidase. However, significant gaps exist in our quantitative understanding of the regulation of NO production in the vascular region. Large apparent discrepancies exist among the published reports that have analyzed the various pathways in terms of the perivascular NO concentration, the efficacy of NO in causing vasodilation (EC₅₀), its efficacy in tissue respiration (IC₅₀), and the paracrine and endocrine NO release. In this study, we review the NO literature, analyzing NO levels on various scales, identifying and analyzing the discrepancies in the reported data, and proposing hypotheses that can potentially reconcile these discrepancies. Resolving these issues is highly relevant to improving our understanding of vascular biology and to developing pharmaceutical agents that target NO pathways, such as vasodilating drugs. *Antioxid. Redox Signal.* 10, 1185–1198.

INTRODUCTION

THE PAST DECADE has seen a wealth of research regarding nitric oxide (NO), a pivotal signaling molecule that regulates blood flow and tissue oxygenation. NO affects two key aspects of O₂ supply and demand: It regulates vascular tone and blood flow by activating soluble guanylate cyclase (sGC) in the vascular smooth muscle, and it controls mitochondrial O₂ consumption by inhibiting cytochrome *c* oxidase. Abnormalities in NO production and transport in vascular systems lead to numerous cardiovascular diseases, including hypertension, atherosclerosis, and angiogenesis-associated disorders (68, 73, 74). A recent review assessed the role of NO regulation of microvascular oxygenation (12).

Endogenous NO is derived from both enzymatic and nonenzymatic sources in and near the vasculature (Fig. 1). The en-

zymatic formation of NO is catalyzed by nitric oxide synthase (NOS) through a series of redox reactions. In this process, L-arginine is degraded to L-citrulline and NO in the presence of molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH). To date, three isoforms of NOS have been definitively identified: neuronal NOS (NOS1 or nNOS), inducible NOS (NOS2 or iNOS), and endothelial NOS (NOS3 or eNOS). NOS1 and NOS3 are constitutive enzymes that are controlled by the availability of intracellular Ca²⁺/calmodulin. NOS2 is an inducible enzyme that is controlled at the level of gene transcription and is expressed in the immune system in response to inflammatory or proinflammatory mediators. NO synthesized by NOS3 in the endothelium has been considered to be the major source of NO for regulating vasoactivity. NO could also be synthesized by mitochondrial nitric oxide synthase (mtNOS), but the existence of mtNOS is still under debate (55).

¹Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, Maryland.

²Department of Physiology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, Virginia.

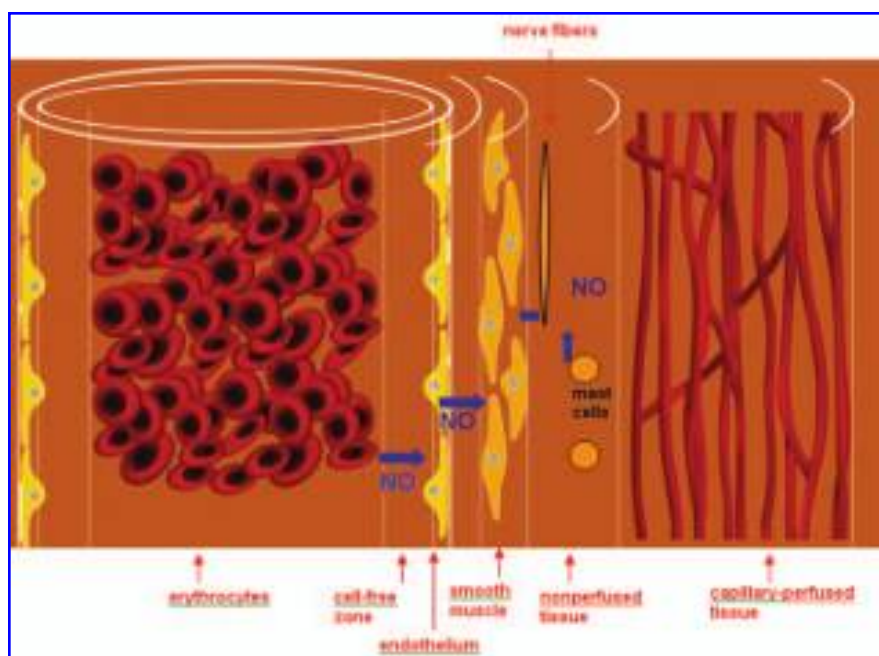


FIG. 1. The distribution of NO in the vasculature. The vasculature consists of the lumen containing erythrocytes, the erythrocyte-free zone resulting from blood flow, the endothelium, smooth muscle, the nonperfused region, and the capillary perfused region. (Adapted from ref. 104.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

In addition to the enzymatic formation of NO catalyzed by NOS, experimental evidence has shown that blood proteins can preserve and release NO bioactivity (64). Furthermore, the nitrite reservoir in the blood and extravascular tissue can be reduced to form free NO under hypoxic or low-pH conditions or both (24, 117). Once formed, NO can be consumed through reactions with a variety of species in the vasculature, such as hemoglobin (to form iron-nitrosyl-hemoglobin or nitrate), O_2 , reactive oxygen species, reactive nitrogen species, sGC, and cytochrome *c* oxidase.

The interactions of NO with sGC and cytochrome *c* oxidase are the focus of this study. We have reviewed the literature and summarized the biochemical pathways that can lead to the release of NO, which can then regulate sGC and cytochrome *c* oxidase activities in the vasculature. Figure 2 outlines these pathways and the NO signaling activities in the immediate vicinity of the vascular wall.

With regard to the regulation of O_2 delivery, NO synthesized by NOS acts as a paracrine regulator of vascular tone, and NO released or converted by circulating blood proteins (*e.g.*, hemoglobin) acts as an endocrine regulator (57, 86). Autocrine regulation of protein *S*-nitrosylation has been recently observed inside endothelial cells (41). It has been well established that NO synthesized by NOS3 in the endothelium is the major contributor to the paracrine regulation of vascular tone and mitochondrial respiration. However, NO from endocrine sources and NOS isoforms other than NOS3 have also been shown to play important physiological roles. In the perivascular region, the inhibition of mitochondrial respiration also involves multiple NO sources. It would be extremely useful to determine quantitatively the contribution of NO from each pathway to its targets (sGC activation and cytochrome *c* oxidase inhibition) under various conditions, particularly normoxia and hypoxia, and to compare them with the NO-concentration measurements in the perivascular region. However, few studies have quantitatively

reviewed the relations among these pathways (see Fig. 2) at the systems biology level.

In this review, we address the following quantitative questions about NO pathways: (a) How much NO is produced from each source in the vasculature, and how are these sources spatially distributed? (b) What is the EC_{50} for NO, the concentration required to achieve half-maximal vasodilation? (c) What is its IC_{50} , the concentration required to inhibit mitochondrial respiration by 50%? (d) Are these values in agreement with *in vivo* perivascular NO measurements obtained by using biosensors? (e) How do the amounts of NO from the paracrine and endocrine pathways compare? In our review of the literature, we found large apparent discrepancies among the published reports analyzing the various pathways in terms of perivascular NO concentration, EC_{50} , IC_{50} , and paracrine and endocrine NO release. Resolving these apparent discrepancies by obtaining reliable quantitative data will provide us with a better understanding of vascular biology and also contribute to the development of pharmaceutical agents that can target NO pathways, such as vasodilating drugs.

VARIATION IN PERIVASCULAR NO CONCENTRATION, C_{NO}

The concentration distribution of NO in the vascular smooth muscle and the perivascular region is a critical parameter for determining the degree of vascular relaxation and tissue oxygenation, and it has, therefore, been extensively studied by using various theoretical and experimental approaches. *In vivo* experimental measurements by using electrochemical approaches, which convert NO presence to electric current, generally report concentrations of perivascular NO (C_{NO}) on the order of several hundred nanomolar (100, 101, 108, 113); a summary of

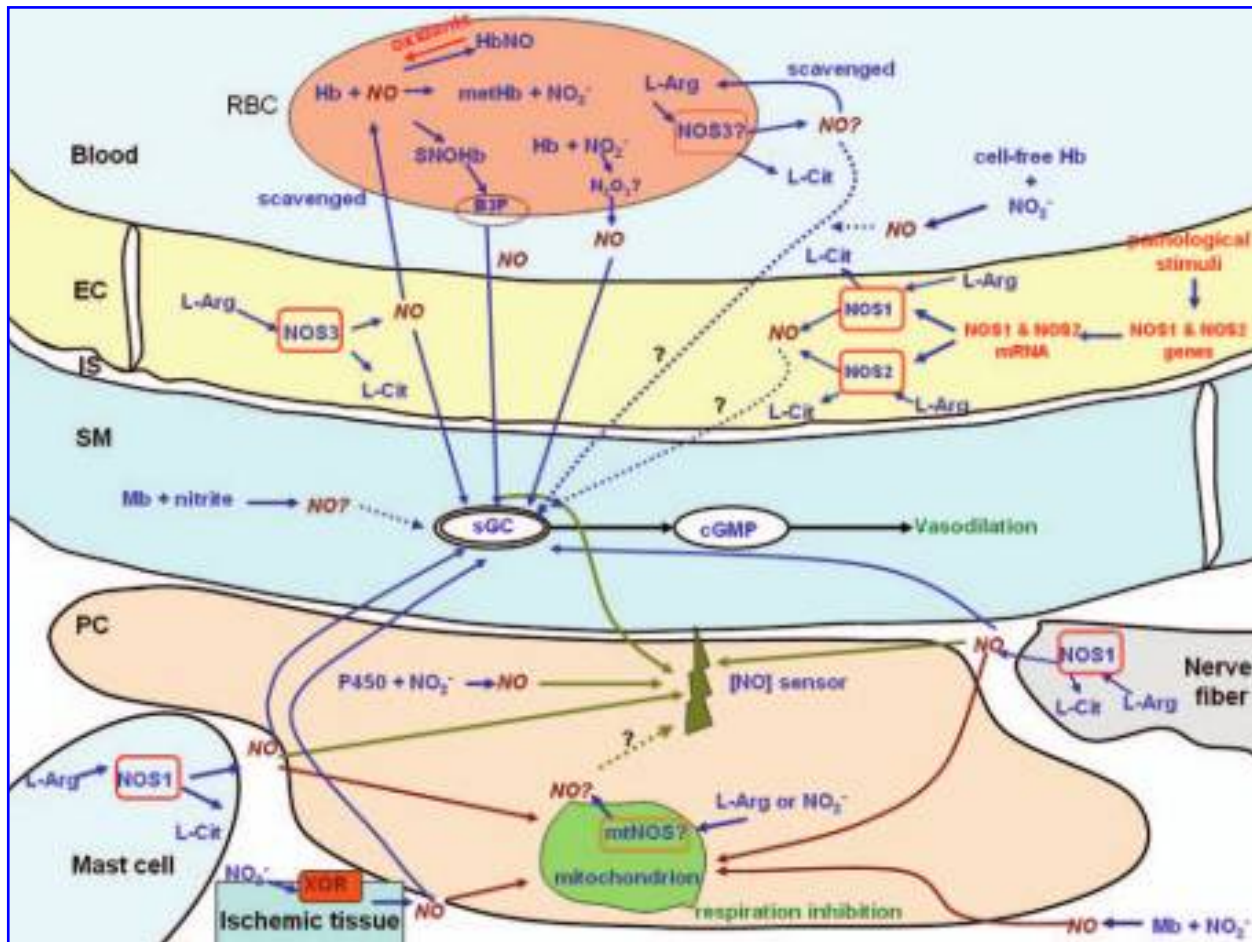


FIG. 2. Major pathways of NO release that can regulate the activities of sGC and cytochrome *c* oxidase in the immediate vicinity of the vascular wall. A variety of enzymatic and nonenzymatic sources of NO can contribute to the NO concentration measured in the perivascular region. [NO] sensor represents a device that can be placed in the perivascular region and measures NO concentration. NO sources by nitric oxide synthase include NOS3 in the endothelium and erythrocytes, NOS1 in the nerve fibers, mast cells, and other tissues, mtNOS in the mitochondria, and NOS2 in a variety of tissues under pathologic conditions. Xanthine oxidoreductase and cytochrome P450 reductase can reduce nitrite to NO, respectively. Nonenzymatic NO release can potentially come from S-nitrosylated blood proteins, nitrite reduction by heme-containing proteins, and iron-nitrosyl-hemoglobin, among other sources. See the text for the details of these pathways. EC, endothelial cell; IS, interstitial space between the endothelium and smooth muscle; PC, parenchymal cell; RBC, red blood cell; Hb, hemoglobin; Mb, myoglobin; B3P, band 3 protein; metHb, methemoglobin; NO₂⁻, nitrite; NO₃⁻, nitrate; L-arg, L-arginine; L-cit, L-citrulline; cAMP, adenosine 3',5'-cyclic monophosphate; cGMP, 3',5'-cyclic guanosine monophosphate; Mt, mitochondrion; XOR, xanthine oxidoreductase; P450, P450 reductase.

these data is given in Table 1. The reported values range from ~200 to 1,000 nM under control conditions. Zhou *et al.* (116) measured a level of 2.6 μM periarterial NO in spontaneously hypertensive rats (as compared with 1.1 μM in their control rats), a level that is, to our knowledge, the highest perivascular NO concentration reported thus far. Note that different NOS expression may be found and, thus, perivascular NO concentration distribution among different hierarchies of vasculature exist (49), but most experimental results on small arterioles (diameter, ~60 μm) point to several hundred nanomolar NO. If the NO is produced primarily in the endothelium, then, considering the geometric structure of the vasculature, it must first cross the vascular smooth muscle before reaching the perivas-

cular region, where the measurements are conducted. Thus, the level of NO in the smooth muscle is presumably higher than, or no less than, that measured *in situ* (see Table 1). How the 200–1,000 nM (or even higher) concentrations of NO in the smooth muscle and perivascular region might regulate blood flow and tissue oxygenation is discussed later.

In addition to experimental approaches, *in silico* modeling has been used to predict the distribution and levels of NO. In 1994, Lancaster (57) constructed a computational model to simulate the diffusion and reaction of NO in a system mimicking the endothelium and its surrounding tissue. Vaughn *et al.* (107) formulated a mathematical model that included the geometric information of endothelial cells and also fitted the experimen-

TABLE 1. EXPERIMENTAL MEASUREMENTS OF PERIVASCULAR NO CONCENTRATIONS

Reference	NO concentration	Animal model	Arteriolar size	Method	Experimental condition
Tsai <i>et al.</i> (2006) (101)	200 nM	50- to 60-g male golden Syrian hamsters; retractor muscle	Diameter, 58 μm	Amperometric biopolymer-coated carbon fiber microelectrodes	<i>In vivo</i> ; superfused with salt solution
Tsai <i>et al.</i> (2006) (100)	600 nM 1,200 nM	55- to 60-g male golden Syrian hamsters; retractor muscle	Diameter, 50–60 μm	Amperometric biopolymer-coated carbon fiber microelectrodes	<i>In vivo</i> ; control samples, high-viscosity plasma
Vukosavljevic <i>et al.</i> (2006) (108)	310–388 nM for small, medium, and larger arterioles	Male Sprague-Dawley 200- to 300-g rats; exteriorized mesentery and intestine	Measured small, medium, and large arterioles	Recessed microelectrodes with Nafion membrane	<i>In vivo</i>
Zani and Bohlen (2005) (113)	545 \pm 144 nM	Male Sprague-Dawley rats; intestine arterioles	Diameter, 53 μm	Recessed-tip glass microelectrode	<i>In vivo</i>
Nase <i>et al.</i> (2003) (71)	397 \pm 26 nM	Male Sprague-Dawley rats; intestine arterioles	Diameter, 52 μm	Recessed-tip glass microelectrode	<i>In vivo</i>
Malinski <i>et al.</i> (1993) (65)	1,300 nM	Adult New Zealand rabbits; thoracic aorta	N/A	Porphyritic electrochemical sensors	<i>Ex vivo</i> ; stimulated by bradykinin

tal findings of Malinski *et al.* (65) to the model. With this model, Vaughn *et al.* estimated the NO production rate per unit area from the endothelium, assuming that all the measured NO was from the endothelial source. Several subsequent models also were based on the measurements of Malinski *et al.* (18) and the estimates of Vaughn *et al.* (20, 21, 29, 42, 47, 48, 105). Generally, these theoretic models predict an \sim 100-nM level of NO near an arteriole. Kavdia and Popel (46) and Lamkin-Kennard *et al.* (56) modeled the effect of other enzymatic NO production included in the simulation, assuming the nonendothelial NO production rates were comparable to that estimated for the endothelium by Vaughn *et al.* (107); in this analysis, several hundred nanomolar NO in the vascular bed was predicted.

Despite this success in measuring NO *in situ* and in modeling NO transport *in silico*, unresolved issues still concern the NO-concentration profile in the vasculature. First, the measured NO concentrations cover a large range, from 200 to $>$ 1,000 nM

under control conditions. This \sim 800-nM variation is huge when compared with the potency of NO with regard to its targets (discussed later), suggesting that the physiological implications of this variation ought not to be ignored if we are to gain a quantitative understanding of the regulation of vasoactivity by NO. These large variations in experimental measurements may reflect either (a) a complex and dynamic distribution of specific NO sources or (b) limitations in the measurement method. Second, computational modeling has been limited in terms of considering a full scenario of NO production under physiologic conditions. Previous computational models have used a high rate of NO production by the endothelium, estimated by fitting the data for perivascular NO concentrations by using a mathematical model (107), with the assumption that NOS3-derived NO from the endothelium is the sole source in the vasculature. However, it has been recognized that a number of biochemical pathways can contribute to the NO presence in the perivascu-

lar region (see Fig. 2). For instance, microfluorographic and immunohistochemical analyses have indicated that strong expression of NOS1 exists around arterioles (38, 45, 98). In addition to the contributions from NOS1 and NOS3, other NOS isoforms in the vasculature, such as NOS2 or the putative mitochondrial nitric oxide synthase (mtNOS) (55), could contribute to the measured perivascular [NO]. Moreover, Kleinbongard *et al.* (51) reported the expression of functional NOS3 on the intraluminal erythrocyte membrane as another potential source of NO within the arteriole. This finding has been confirmed by others (109), but Hilarius *et al.* (36) reported finding little, if any, NOS3 from this source. Furthermore, the endocrine signaling from blood-borne NO bioactivity (*e.g.*, *S*-nitrosohemoglobin and nitrite) has been proposed to be an important mechanism for matching blood flow to the metabolic activities of local tissues (24, 64, 117). However, little is known about NO production rates within specific cellular elements around an arteriole.

One may ask whether it is possible to predict the *in vivo* NO distribution by combining *in vitro* NO release studies and *in silico* simulations, because it is difficult to obtain NO release rates directly *in vivo*. However, the reported values for NO release from cultured endothelial cells cover a large range as well. Arnal *et al.* (4) measured the accumulation of nitrogen oxides (nitrite plus nitrate) from cultured bovine aortic endothelial cells. From their experiments, the basal NO production was calculated to be 0.04 $\mu\text{M}/\text{sec}$; the value reported by Kuchan and Frangos (54) from cultured human umbilical vein endothelial cells (HUVECs) was 0.21 $\mu\text{M}/\text{sec}$ in the absence of a shear-stress stimulus and was 10.4 $\mu\text{M}/\text{sec}$ when a shear stress of 6 dyn/cm^2 was applied. Hood *et al.* (37) measured the nitrite/nitrate accumulation over time from cultured HUVECs, and the NO production rate from their study was 0.24 $\mu\text{M}/\text{sec}$ under control conditions. Moreover, it is possible that the *in vitro* measurements are complicated by the fact that other isoforms of NOS may be expressed in endothelial cells as a result of adaptive immune responses (25).

Although a variety of NO-sensing techniques, such as chemiluminescence, spectrophotometry, electron paramagnetic resonance (EPR), and electrochemical sensors (28, 79, 103, 110), are available, few are considered to be reliable *in vivo*, and fewer still permit wide-field temporal imaging. For instance,

EPR spin-trapping and conventional fluorometric imaging suffer from low spatial resolution and nonspecific interactions that lead to unclear data (69). NO microelectrodes lack specificity as well. Diaminofluoresceins (DAF-2 and subsequent derivatives such as DAF-FM), a class of weakly fluorescent compounds that increase their fluorescence quantum efficiency by >100-fold after reacting with NO-derived species (*i.e.*, nitrosonium ion), seem to allow temporal-spatial mapping of NO gradients in the microvasculature. However, their application to obtaining quantifiable data has been limited to *in vitro* measurements because of variations in the *in vivo* cellular topography and composition, leading to uneven dye absorption (or loading in the case of the diacetate form), nonuniform concentrations, and a poor correlation between NO levels and the increase in fluorescent intensity. In addition, these dyes are susceptible to nonspecific reactions with physiologic products such as peroxynitrite and ascorbic acid (114).

NO CONCENTRATION AND EC₅₀

In the vascular smooth muscle, NO activates sGC and causes vasodilation. It has been established that the activation of sGC by NO proceeds *via* a two-step mechanism: Initial NO binding to sGC partially activates this enzyme through the formation of a six-coordinate nitrosyl intermediate, and the subsequent conversion of this species to a five-coordinate nitroxyl complex occurs through NO-dependent or NO-independent pathways (104, 115). However, the EC₅₀ for NO, the concentration required to achieve half-maximal sGC activation, has not yet been fully established. Clear answers to fill this gap in our knowledge are of great importance for the development of vasodilating drugs.

Studies addressing this problem have been conducted at both the molecular biology and physiology levels and by computational modeling. We have compiled the available information regarding the potency of NO in activating sGC or inducing vasodilation (Table 2). Several studies (5, 83, 95), including those that observed an interaction between NO and purified sGC, have shown that a high level of NO (250–1,600 nM) is required to produce half-maximal activity of the enzyme. Other experi-

TABLE 2. A SURVEY OF EC₅₀ VALUES FOR THE POTENCY OF NO IN sGC ACTIVATION

Reference	EC ₅₀ (nM)	Method
Stone and Marletta (1996) (95)	250	<i>In vitro</i> ; interaction of NO with purified sGC
Russwurm <i>et al.</i> (1998) (83)	690	<i>In vitro</i> ; interaction of NO with purified sGC
Bellamy <i>et al.</i> (2000) (9)	20	<i>In vitro</i> ; interaction of NO with purified sGC in cerebellar cells
Artz <i>et al.</i> (2001) (5)	1,600	<i>In vitro</i> ; interaction of NO with purified sGC
Condorelli and George (2001) (23)	23	<i>In silico</i> ; computational simulation of the kinetics of sGC activation by NO
Bellamy <i>et al.</i> (2002) (8)	3.9	<i>In vitro</i> ; interaction of NO with sGC in brain tissue
Roy and Garthwaite (2006) (82)	10	<i>In vitro</i> ; interaction of NO with sGC in cerebellar cells and platelets
Rodriguez-Juarez (2007) (81)	2.9	<i>In vitro</i> ; interaction of NO with sGC in tetracycline-inducible HEK-293 cells
Yan <i>et al.</i> (2007) (112)	9.7	<i>Ex vivo</i> ; apparent vasorelaxation of rat thoracic aorta in phosphate buffer

ments (9, 81, 82) that have characterized the NO-sGC interaction in the cellular environment or with the use of purified enzyme exposed to endogenous NO have yielded much smaller EC_{50} values (2.9–20 nM). Condorelli and George (23) conducted an *in silico* study by analyzing the kinetics of sGC and found the EC_{50} to be ~23 nM. Yan *et al.* (112) measured rat aortic relaxation as a function of NO concentration *ex vivo* and found that half-vessel relaxation could be induced by 9.7 nM NO in phosphate buffer. Also, as pointed out by these authors, because some of the 9.7 nM NO in the buffer must be consumed before reaching the target in smooth muscle, the real EC_{50} should be smaller than this value.

We have discussed the perivascular NO concentration measurements (see Table 1) that are in the range of 200–1,000 nM, or even higher. These studies have left us with an apparent paradox: On the one hand, the perivascular values for the concentration of NO as measured *in vivo* by NO microelectrodes have been generally reported as several hundred nanomolar under control conditions, and, in the interpretation of the results, most of the NO available to the smooth muscle has been attributed solely to endothelial sources; on the other hand, although variations are found in the EC_{50} values reported in the literature (see Table 2), studies in the cellular environment and using endogenous NO as the source usually point to levels <10 nM (81, 112). Furthermore, as suggested by Kollau *et al.* (53), concentrations <1 nM could trigger maximal vascular relaxation through a mechanism that does not require the full potential of 3',5'-cyclic guanosine monophosphate (cGMP) accumulation achievable with full cyclase activation.

Thus, a review of the literature raises an important question: If the EC_{50} is indeed as low as the value quoted earlier (several nanomolar), and all the perivascular NO is derived from endothelium and is in the range of several hundred nanomolar, smooth muscle would be constantly relaxed. How, then, is the vasculature regulated? At present, this question remains unanswered. Although arteriolar dilation can be caused by a number of other factors (*e.g.*, conducted responses and endothelium-derived hyperpolarizing factors), and vasodilation can be counteracted by constrictors (*e.g.*, endothelin-1), a large apparent discrepancy exists between how much NO is present and how powerful it appears to be.

It should be noted that the NO-mediated regulation of sGC in the smooth muscle can proceed by both amplitude- and frequency-dependent mechanisms (90, 104). Tonic NO, reflecting a continuous low-level production of NO, forms a stable and low-activity sGC heme complex, whereas acute production of NO transiently and fully activates this NO-bound sGC (15). Transient NO release could represent a more efficient means of activating sGC, and it can increase cGMP formation severalfold. Thus, the frequency of NO bursts may limit cGMP formation and regulate vascular tone (104). Clearly, a study that will accurately determine the NO production from specific sources in the vasculature and assess its effect on sGC activation is desirable.

NO CONCENTRATION AND IC_{50}

In addition to its role in regulating vascular tone to increase O_2 delivery, NO competes with O_2 for binding to cytochrome

c oxidase in the mitochondria of the extravascular tissue and causes a substantial inhibition of tissue respiration. As a result, the K_m value for tissue O_2 consumption is strongly dependent on the local NO concentration. The decreased oxygen consumption in the vicinity of the blood vessel can facilitate the delivery of the O_2 supply to more hypoxic tissue. Furthermore, the NO production (and thus NO distribution) is a function of PO_2 : enzymatic NO production is attenuated as the oxygen substrate supply decreases, whereas nonenzymatic NO production (*e.g.*, reduction of extravascular nitrite under low pH and/or low O_2 conditions) is expected to increase (39, 80, 91). Thus, a mutual interdependence of NO release and O_2 delivery is found.

The interplay of NO and O_2 in the perivascular region is even more complicated because the IC_{50} of NO also depends on the O_2 concentration. The apparent K_m value of tissue respiration is a function not only of the NO concentration but also of the concentration of ambient O_2 : Half-inhibition of tissue O_2 consumption in brown adipose tissue occurs at 364 nM NO with 180 μM O_2 , 69 nM NO with 72 μM O_2 , and 11 nM NO with 32 μM O_2 , with respect to their values at 0 nM NO (52). Different values of IC_{50} have also been reported in other studies. Rodriguez-Juarez (81) and Bellamy *et al.* (8) reported IC_{50} values for NO of 141 nM in tetracycline-inducible HEK-293 cells and 120 nM in cerebellar cells, respectively, with 30 μM O_2 . Basically, under different O_2 conditions, the inhibitory effect of the same amount of NO on cytochrome *c* oxidase is different (11, 58): Under hyperoxic conditions, cytochrome *c* oxidase activity is relatively insensitive to NO regulation, and its inhibition requires a high concentration of NO, far larger than that required to interact with sGC; under normoxic conditions, cytochrome *c* oxidase activity becomes more sensitive to this signaling molecule, but the IC_{50} is still fairly large; and under hypoxic conditions (such as tissue oxygenation being reduced to <30 μM), an even lower amount of NO can regulate mitochondrial respiration. It is worth noting that the tissue oxygen concentration, depending on the specific tissue and vascular bed, varies from ~35–120 μM (20–70 mm Hg) (10, 34, 72) and, thus, the hypoxic condition herein refers to the relative hypoxia at which an inadequate O_2 supply to tissues occurs.

It is important to note that the perivascular NO concentrations (200–1,000 nM, as mentioned earlier) are generally higher than the IC_{50} values, particularly at low PO_2 [*e.g.*, 30 μM O_2 , an oxygenation condition close to physiologic conditions in certain tissues (72)]. Whereas the discrepancy between the IC_{50} and C_{NO} is not as large as that for its EC_{50} , a C_{NO} of several-hundred nanomolar would still inhibit most of the cellular respiration and leave little room for any control of O_2 delivery through NO-dependent inhibition of mitochondrial respiration. This discrepancy at least exists in the region near the arteriole, where it is not perfused by erythrocyte-containing capillaries, which represent a significant NO sink and can cause a sharp decrease in NO availability. Thus, perhaps a heterogeneous distribution of NO in the microvasculature is determined by its specific sources and has a strong influence on the O_2 -concentration distribution. It has recently been suggested that under hypoxic conditions, nitrite, which can induce vasodilation through a NO-generating mechanism reduced by deoxygenated hemoglobin (24) or independent of nitrite reductase activities (27), can also react with the myoglobin that is found in skeletal muscle and also in smooth muscle (77). This reaction

releases NO, which partially inhibits respiration in the adjacent tissue and ensures that more O₂ will be delivered to the more hypoxic tissue (88). Conversely, the cellular consumption of NO itself in the parenchymal tissue is a function of both NO and O₂ concentrations (99).

In addition to regulating respiration in the extravascular tissue, NO seems to be able to modulate tissue oxygenation by reducing O₂ consumption by the vessel walls (14, 87). According to some reports, large arteriolar transmural Po₂ gradients are attributable to the large amount of O₂ consumed by the microvascular wall, orders of magnitude greater than that consumed by the surrounding tissue (102). This high O₂-consumption rate can be regulated by NO: An increase in the local NO concentration can reduce the rate of O₂ consumption by the vascular wall, facilitating the delivery of O₂ to the surrounding hypoxic tissue; a decrease in the local NO concentration produces the opposite outcome. However, this hypothesis has been questioned by a number of studies. We and others have shown that it is unlikely that the thin endothelium has sufficient metabolic activity to consume three orders of magnitude more O₂ than the surrounding tissue (50, 106, 111). Also, recent Po₂-profile measurements near arterioles in rat mesentery by using a scanning phosphorescence-quenching microscopy technique found no steep Po₂ gradient across the vascular wall (34). The authors of this study proposed that the measured higher-than-expected O₂ consumption by the endothelium (14, 87, 102) may be an artifact produced by the O₂ consumption by phosphorescence quenching in the interstitial space, where the consumed O₂ cannot be replenished as quickly as it is in the flowing blood.

As previously noted, NO regulates O₂ delivery through binding to its targets, sGC and cytochrome *c* oxidase. sGC is located in the vascular smooth muscle, whereas cytochrome *c* oxidase is located in the perivascular region. However, the sensitivities of these two targets to NO differ by 50-fold (IC₅₀: 141 nM; EC₅₀: 2.9 nM) at the 30-μM O₂ level (81). Given these

relatively low EC₅₀ and high IC₅₀ values, if all the measured NO in the perivascular region (see Table 1) were derived from the endothelium, the NO-sGC-cGMP pathway in smooth muscle would be saturated before NO from this source would exert any regulatory effect on the mitochondrial respiration in parenchymal cells. Thus, these findings add to the NO paradox and further encourage us to hypothesize that (a) multiple sources contribute to the NO in the perivascular region, and (b) a heterogeneous NO distribution is of importance in regulating microvascular oxygen delivery.

SOURCES OF VASCULAR AND PERIVASCULAR NO

Although the endothelium is considered the major NO-producing source for regulating vascular tone and tissue oxygenation (68), a number of sources of NO have been proposed, in addition to the endothelial source NOS3 in the arteriolar wall. Figure 2 and Table 3 present the results of our survey of the studies addressing possible NO sources, both enzymatic and nonenzymatic, in the vasculature and perivascular tissue. All of these sources could contribute to the measured NO concentration listed in Table 1.

In addition to the NOS3 in the endothelium, this form of the enzyme is also expressed in functional form in erythrocytes (51). Erythrocytes are rich in hemoglobin, which is a potent NO scavenger. The NO level resulting from intravascular nitric oxide is too low to have any physiological effect if free diffusion is the transport mechanism involved (61). It appears that most of the NO that is produced by this source through complex biochemical reactions involving multiple substrates and coenzymes would be immediately converted to end-metabolic products unless a protective mechanism existed to allow the NO that is formed to escape being scavenged. How much this source

TABLE 3. NO SOURCES IN THE VASCULATURE

<i>Source</i>	<i>Location</i>	<i>O₂ condition to function</i>
Hydroxylation of L-arginine by NOS1	Nerve fibers; mast cells (45)	Sensitive to O ₂ (high K _m) (30, 96)
Hydroxylation of L-arginine by NOS2	Macrophages; aged endothelium; many tissues under inflammatory conditions (70, 84)	Sensitive to O ₂ (relatively high K _m) (96)
Hydroxylation of L-arginine by NOS3	Vascular endothelium; erythrocytes (51, 68)	Insensitive to O ₂ (low K _m) (78)
Nitrite reduction by NOS3	Endothelium (33)	NO released during anoxia (33)
mtNOS	Parenchymal tissues (55)	N/A
S-nitrosohemoglobin	Erythrocytes (64)	NO released during hypoxia (91)
S-nitroso-glutathione or S-nitrosoalbumin	Plasma (32)	NO released during hypoxia (32)
Nitrite reduction by hemoglobin	Erythrocytes, plasma (39)	NO released during hypoxia (39)
Nitrite reduction by myoglobin	Skeletal muscle and myocardial tissue (88)	NO released during hypoxia (88)
Nitrite reduction by xanthine oxidoreductase	Ischemic tissues (60, 62)	NO released under ischemic or anaerobic conditions (60, 62)
Nitrate and nitrite reductions by cytochrome P450 reductase and cytochrome P450	A variety of tissues (26, 59)	N/A

K_m refers to the Michaelis-Menten constant.

contributes to vasodilation and other physiological functions (*e.g.*, regulating erythrocyte deformability) is not yet clear.

Recent microfluorographic and immunohistochemical analyses have provided a qualitative indication that in addition to NOS3, strong expression of NOS1 around arterioles exists: Kashiwagi *et al.* (45, 97) measured the NO distribution around the microvessels by using a fluorescence-detection technique coupled with specific NOS inhibitors and of NO produced by NOS1 in the nerve fibers and mast cells. This finding was also confirmed by their immunohistochemical staining to detect NOS isoforms in various tissues. Evidence also suggests that NOS1 can compensate for the loss of NOS3 in NOS3-knockout mice and thus induce a change in NOS expression. Studies in NOS3-knockout mice (38, 98) have shown that NOS1-derived NO and prostaglandins can maintain flow-induced vasodilation; immunohistochemical measurements also showed the presence of NOS1 in vascular tissue in wild-type mice and in the endothelium of the coronary arteries in NOS3-knockout mice. Mathematical models (46, 56) suggest that NOS1 (or NOS2) in the perivascular region can make a significant contribution to the perivascular NO distribution because it is located farther from the lumen than the NOS3 in the endothelium, thereby preventing the scavenging of the NO by hemoglobin. We have formulated a molecular-level theoretic/computational model based on the analysis of the biochemical pathways of NOS1 and NOS3 that has allowed us to quantify the NO production in the microvasculature. We have obtained paradoxical results, in that the NO derived from NOS3 could not account for the reported perivascular NO concentration (17, 18). The predicted NO release rates from NOS3 in the endothelium were in the range of $5\text{--}17 \times 10^{-3} \mu\text{M}/\text{sec}$ (17), and those from NOS1 in the nerve fibers and mast cells in the perivascular region were in the range of $0.39\text{--}1.16 \mu\text{M}/\text{sec}$ (18); using these values gave NO concentrations at the arteriolar smooth muscle of ~ 0.1 and 5 nM , respectively. In our models, we specifically considered the catalytic activities (measured *in vitro*), amounts, and locations of NOS1 and NOS3.

These paradoxical predictions led us to investigate the amount of NO from each identified source and how each one specifically regulates its target (*i.e.*, sGC and cytochrome *c* oxidase). However, a number of questions remain to be answered regarding the role of NOS1 in vasodilation. In NOS3-knockout animal models, hypertension or increased vascular tone has been observed, and the mechanotransduction-related production of NO (likely the results of an increasing influx of Ca^{2+} into the endothelial cells) and subsequent vasodilation of arterioles have also been documented in a variety of studies (6, 22, 75, 100). The conclusion that NOS1 is the major source of NO regulating vascular tone seems contradictory to those findings that closely link the regulation of vascular tone to NOS3 activity. Experiments featuring high-specificity approaches to real-time detection of NO-derived reactive species (such as 4,5-diaminofluorescein diacetate with a calibrated signal and the use of selective NOS inhibitors) would be very helpful in delineating the contribution of each NOS isoform.

The inducible form of NOS, or NOS2, can catalyze NO formation at a faster rate than can other NOS isoforms under the same conditions (96). NOS2 is expressed in macrophages and a variety of tissues under inflammatory conditions. Berkowitz and colleagues (84) also detected NOS2 expression in the en-

dothelium of aged rats and showed that it serves as a source of *S*-nitrosylation for a variety of proteins (84). Moreover, the putative mtNOS could produce NO from the parenchymal cells, but, as discussed earlier, its existence is still under debate. Other enzymes that can produce NO include xanthine oxidoreductase in mammalian cells, which can reduce nitrite to NO *via* an acid-catalyzed mechanism, particularly in ischemic tissues (60, 62), and cytochrome P450 reductase and cytochrome P450, which can reduce nitrate to nitrite and nitrite to NO, respectively (26, 59).

Nonenzymatic NO production can also be a significant source of NO under certain conditions: *S*-nitrosylated blood proteins and peptides, such as hemoglobin and glutathione, are thought to serve as a storage pool for NO, and NO can be released when a reduction in P_{O_2} is sensed (91). Note that the SNOHb-induced vasodilation could be through an NO-independent mechanism (92). Also, NO can be stored in the form of circulating nitrite, which can be reduced to NO under low-pH conditions (117) by deoxygenated hemoglobin in the lumen (39), or by deoxygenated myoglobin in the tissue (88). It is interesting to note that nitrite-dependent hypoxic vasodilation can be independent of the NO-generating pathway by nitrite reductases (27). In addition to hemoglobin, NOS3, which normally requires O_2 as a substrate to produce NO, can react with nitrite under anoxic conditions, releasing NO (33). Furthermore, iron-nitrosyl-hemoglobin (HbNO), which is a relatively stable species as the product of NO and deoxyhemoglobin, can release the NO molecules at a significantly high rate in the presence of oxidants (89). Blood-borne NO bioactivity has been proposed to be a key mechanism for matching blood flow to the metabolic activity of local tissue.

Despite intense investigation of the endocrine NO reservoir as a vasodilator, little quantitative knowledge is available concerning the transport and concentration distribution from such a reservoir. This quantitative question has presented two conceptual problems: whether the NO from intraluminal sources is sufficient to induce vascular smooth muscle relaxation through the known NO-sGC-cGMP pathway, and whether it is comparable to the paracrine source of NO (61). Because of the complex biochemical reactions involved in NO release and the difficulty in discriminating the NO that might come from other enzymatic or nonenzymatic sources, no direct *in vivo* experimental measurements have been made of the amount of NO released from these pathways.

Computational modeling has been used to predict NO release from certain intraluminal pathways. By using a multicellular computational model simulating NO release from erythrocytes and assuming that a membrane-associated mechanism facilitates NO export out of these cells or a nonreactive intermediate species, we predicted that $0.25\text{--}6 \text{ pM}$ or $\sim 40 \text{ pM}$ NO is delivered by intraerythrocytic *S*-nitrosohemoglobin (SNOHb) to vascular smooth muscle (19). The nitrite reduction by hemoglobin has also been quantified by modeling: Jeffers *et al.* (43) calculated that 0.08 pM NO is present in smooth muscle, assuming that free diffusion is the only transport mechanism. Our computational simulations confirmed a similar amount of NO delivery with free diffusion, whereas $\sim 40\text{--}260 \text{ pM}$ could be contributed by this source to smooth muscle, provided that a protected mechanism or a nonreactive intermediate species exists (16). The model predictions remain to be validated exper-

imentally when more-sophisticated approaches for detecting NO from different pathways become available.

Figure 2 shows the biochemical pathways that can lead to NO release in the vasculature and contribute to the NO-dependent regulation of sGC and cytochrome *c* oxidase activities. A quantitative analysis of the contribution made by each pathway is needed to address the complex and dynamic nature of NO signaling from a variety of specific sources, giving due consideration to their spatial distribution. A combination of experimental and computational approaches, including immunohistochemistry, *in situ* DAF-2T fluorescence for the detection of NO-derived reactive species, and computational analysis of biochemical pathways leading to NO release, could reveal with high resolution the spatial distribution of significant sources of NO.

PARACRINE OR ENDOCRINE REGULATION

Cytochrome *c* oxidase activity is regulated mainly through a paracrine mechanism: NO produced locally by various sources (except mtNOS) diffuses to the targets in the perivascular region and inhibits mitochondrial respiration. In addition, an autocrine regulation pathway may be involved if putative mtNOS does indeed exist.

The regulation of NO in vascular smooth muscle appears to be more complicated. Based on his computational analysis of NO diffusion and reactions, Lancaster (57) pointed out that the NO produced in endothelial cells primarily conducts paracrine signaling in the vasculature, and thus the spatial relations between the specific sites of NO formation and the neighboring targets of this NO are of great importance. Because of the close proximity of the endothelium to the blood vessel lumen, most of the NO that is locally produced by endothelium-based NOS3 was initially thought to be readily consumed because of the massive presence of hemoglobin in the lumen (*i.e.*, as a “cost” of paracrine signaling). Subsequent studies have shown, however, that the encapsulation of hemoglobin by the erythrocytic membrane and the existence of the cell-free region (shown in Fig. 1) in the blood can prevent, to a large extent, this NO from being consumed (13, 29, 67). Still, a large amount of NO derived from NOS3 is converted to inactive metabolic products (*e.g.*, nitrate) by hemoglobin through dioxygenation and iron-nitrosylation reactions (44).

In addition to its paracrine mechanism, NO seems to mediate endocrine signaling: NO bioactivity is preserved in other more-stable species that circulate in the blood and is released under conditions of hypoxia, when vasodilation is needed most. The SNOHb theory is one of the major hypotheses to account for the endocrine signaling: According to this hypothesis, in addition to reacting with the ferrous or ferric heme of hemoglobin, NO can be transferred from the heme iron of hemoglobin to the cysteine residue at position 93 on the β chain to form SNOHb, which is relatively stable and can convey NO bioactivity to hypoxic tissues (64). Other blood proteins or peptides that can be *S*-nitrosylated, such as glutathione, can potentially act as NO preservers as well (91, 93). As discussed earlier, NO delivered to smooth muscle through this pathway is calculated

to be at the 0.25–6 pM or ~40 pM level, depending on the possible NO-transport mechanisms involved (19).

A competing hypothesis addresses the role of nitrite anion in endocrine signaling by NO. Nitrite, formed as one of metabolic products of enzymatic NO or obtained as a dietary supplement, is found nearly to double human forearm blood flow at a high dose (~200 μ M) and to have an NO-dependent vasodilating effect at a near-physiologic concentration level (40). The molecular mechanism for this nitrite reduction involves the reducing activities of heme-containing globins, such as hemoglobin or myoglobin, to release NO (39, 88). The resulting NO may be exported out of the lumen as the intermediate reaction product, likely in the form of nitrous anhydride (N₂O₃), as proposed by Robinson and Lancaster (80) and recently experimentally confirmed by Basu *et al.* (7). NO from the nitrite reservoir in the lumen is calculated to be ~40–260 pM under physiologic conditions, depending on the protected mechanisms of NO transport (16). Interestingly, the study by Angelo *et al.* (3) suggested a possible link between these two hypotheses: Hemoglobin could synthesize SNOHb, by using nitrite as a substrate (3). Furthermore, experimental evidence has shown that adenosine 5'-triphosphate (ATP) can be released from erythrocytes after O₂ reduction is sensed, leading to vasodilation produced by inducing a conducted vasomotor response or stimulating NOS catalytic activity (31, 94).

Thus, a growing body of evidence suggests that in addition to its paracrine regulation by NO that has been locally produced by NOS, vascular tone can be regulated through an endocrine effect produced by NO released from circulating erythrocytes after sensing low Po₂. It is likely that paracrine NO is responsible for the basic homeostasis under resting conditions, whereas endocrine NO contributes to hypoxic vasodilation, when an increase in metabolic activity (such as exercise) consumes a large amount of O₂ and leads to a decrease in the tissue Po₂. The change in O₂ level results in vasodilation, which increases the blood flow and ensures the delivery of the adequate O₂ to match the tissue's metabolic requirements.

How does the vasculature sense O₂, and what type of regulation becomes dominant when this process occurs? Although our understanding is still incomplete regarding the quantities of NO resulting from each specific pathway, it appears that local production of paracrine NO is higher than that of NO from endocrine sources in the lumen (18–20, 47, 101). It is reasonable to postulate that the paracrine regulation of vascular tone by NOS-derived NO is significantly attenuated under conditions of low O₂ tension, when NO from erythrocytes becomes an important source. The NO that is locally produced by NOS3 has been thought to act as a paracrine source to regulate the vascular tone; however, the K_m value of NOS3 for O₂ has been reported to be ~7 μ M (78), a level much lower than the O₂ concentration in the precapillary arterioles (76). Thus, an apparent contradiction exists between endocrine NO acting as the major vascular relaxing factor under conditions of hypoxia and the low sensitivity of NOS3 to the change in O₂ concentration. One possible explanation for this paradox is that other isoforms of NOS with higher K_m values contribute significantly to this paracrine regulation and sense the change in ambient O₂ (18).

marily designed to quantify NOS3 expression in cells, they both point to an intracellular concentration of <50 nM. Also, their values are in good agreement with that from the manual for the NOS3 Quantikine Immunoassay Kit (R&D Systems, Minneapolis, MN), which quotes NOS3 concentrations of 5,137 pg/10⁶ ECs (97 nM) for HUVECs and 1,396 pg/10⁶ ECs (26 nM) for human microvascular endothelial cells. These low NOS3 expression values yield low NO production rates from the endothelium (17). Our model would require a 1,000-fold higher NOS3 concentration as an input to yield a NO production rate that would result in an NO concentration in the perivascular region (see Table 1). Here we used an average intracellular NOS3 concentration estimated from a large number (10⁵ or 10⁶) of endothelial cells. However, the distribution of NOS3 may be heterogeneous in different tissues or among endothelial cells from different vessels. Thus, detailed measurements of the NOS3 distribution among the different hierarchies of vasculature could provide a better understanding of NO production in different tissues.

We also applied the same biochemical-pathway analysis to examine the NO release by NOS1 and the contribution from this source to the perivascular NO concentration. Our model of NOS1 catalytic activity and its distribution show that mast cells and nerve fibers can significantly contribute to the perivascular C_{NO} (at levels of ~ 50 nM), but the predicted value still falls short of the experimentally measured values (18). This result points to a higher abundance of NOS1 or NOS3 and/or the existence of other enzymatic or nonenzymatic sources of NO in the microvasculature. Also, it should be noted that a limitation exists for using *in vitro* enzymatic parameters of NOS isoforms in theoretic modeling. NOS activities in intact cells could be different than those reported from the isolated enzyme *in vitro*. The posttranslational modifications or intracellular protein-protein interactions may have an important effect on regulatory control of NO generation, which requires further studies.

CONCLUSIONS

The relation between NO availability and the balance between O₂ supply and demand, especially under hypoxic conditions in precapillary regions, has been extensively studied in the past several years. However, significant gaps remain in our quantitative understanding of the regulation of NO production in the vascular region. Quantitative discrepancies in NO concentrations and activity have presented a conceptual problem in the biology of NO signaling. We have reviewed the NO literature, addressing the NO-concentration levels at various scales, we have identified and analyzed the discrepancies in the reported data, and we have proposed hypotheses that can potentially reconcile these seemingly contradictory data. Studies providing answers to the questions that remain will contribute to a better understanding of NO-production mechanisms and microvascular O₂ delivery, a major problem in cardiovascular physiology.

Discovering discrepancies between theory and experiment that cannot be reconciled, despite adjusting parameters within the physiologic range, is a powerful motivation for further studies and is the major value of theoretical models. Such discrepan-

cies suggest either the existence of experimental artifacts or the need to formulate new hypotheses that form the foundation of the model. This approach is a paradigm for making new discoveries, and its value has been demonstrated time and time again in combined experimental and theoretic studies. We recognize that the modeling approaches that we used may be simplified and must be refined in the future when more-accurate measurements on certain parameters are available. However, the seemingly large discrepancies that have been identified, together with theoretic considerations, have led us to examine the NO sources and their potential heterogeneous distribution in the vasculature and their roles in regulating microvascular O₂ delivery.

ABBREVIATIONS

DAF, diaminofluorescein; EPR, electron paramagnetic resonance; H₄B, tetrahydrobiopterin; HUVEC, human umbilical vein endothelial cell; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOS, nitric oxide synthase; sGC, soluble guanylate cyclase; SNOHb, S-nitrosohemoglobin.

ACKNOWLEDGMENTS

We thank Dr. Dan E. Berkowitz, Dr. Alan N. Schechter, Dr. Barbora Piknova, Dr. Feilim MacGabhann, Dr. Amina A. Qutub, and Emmanouil D. Karagiannis for helpful discussions, and Dr. Nikolaos M. Tsoukias for his contribution to the design of Fig. 1. This study is supported by NIH grants R01 HL018292 and R01 HL079087.

REFERENCES

1. Alderton WK, Cooper CE, and Knowles RG. Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357: 593–615, 2001.
2. Andersen MR, Walker LR, and Stender S. Reduced endothelial nitric oxide synthase activity and concentration in fetal umbilical veins from maternal cigarette smokers. *Am J Obstet Gynecol* 191: 346–351, 2004.
3. Angelo M, Singel DJ, and Stamler JS. An S-nitrosothiol (SNO) synthase function of hemoglobin that utilizes nitrite as a substrate. *Proc Natl Acad Sci U S A* 103: 8366–8371, 2006.
4. Arnal JF, Clamens S, Pechet C, Negre-Salvayre A, Allera C, Girolami JP, Salvayre R, and Bayard F. Ethinylestradiol does not enhance the expression of nitric oxide synthase in bovine endothelial cells but increases the release of bioactive nitric oxide by inhibiting superoxide anion production. *Proc Natl Acad Sci U S A* 93: 4108–4113, 1996.
5. Artz JD, Toader V, Zavorin SI, Bennett BM, and Thatcher GR. In vitro activation of soluble guanylyl cyclase and nitric oxide release: a comparison of NO donors and NO mimetics. *Biochemistry* 40: 9256–9264, 2001.
6. Bagi Z, Frangos JA, Yeh JC, White CR, Kaley G, and Koller A. PECAM-1 mediates NO-dependent dilation of arterioles to high temporal gradients of shear stress. *Arterioscler Thromb Vasc Biol* 25: 1590–1595, 2005.
7. Basu S, Grubina R, Huang J, Conrad J, Huang Z, Jeffers A, Jiang A, He X, Azarov I, Seibert R, Mehta A, Patel R, King SB,

- Hogg N, Ghosh A, Gladwin MT, and Kim-Shapiro DB. Catalytic generation of N(2)O(3) by the concerted nitrite reductase and anhydrase activity of hemoglobin. *Nat Chem Biol* 3: 785–794, 2007.
8. Bellamy TC, Griffiths C, and Garthwaite J. Differential sensitivity of guanylyl cyclase and mitochondrial respiration to nitric oxide measured using clamped concentrations. *J Biol Chem* 277: 31801–31807, 2002.
 9. Bellamy TC, Wood J, Goodwin DA, and Garthwaite J. Rapid desensitization of the nitric oxide receptor, soluble guanylyl cyclase, underlies diversity of cellular cGMP responses. *Proc Natl Acad Sci U S A* 97: 2928–2933, 2000.
 10. Boegehold MA and Johnson PC. Periarteriolar and tissue PO₂ during sympathetic escape in skeletal muscle. *Am J Physiol* 254: H929–H936, 1988.
 11. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome *c* oxidase. *Biochim Biophys Acta* 1504: 46–57, 2001.
 12. Buerk DG. Nitric oxide regulation of microvascular oxygen. *Antioxid Redox Signal* 9: 829–843, 2007.
 13. Butler AR, Megson IL, and Wright PG. Diffusion of nitric oxide and scavenging by blood in the vasculature. *Biochim Biophys Acta* 1425: 168–176, 1998.
 14. Cabrales P, Tsai AG, and Intaglietta M. Nitric oxide regulation of microvascular oxygen exchange during hypoxia and hyperoxia. *J Appl Physiol* 100: 1181–1187, 2006.
 15. Cary SP, Winger JA, and Marletta MA. Tonic and acute nitric oxide signaling through soluble guanylate cyclase is mediated by nonheme nitric oxide, ATP, and GTP. *Proc Natl Acad Sci U S A* 102: 13064–13069, 2005.
 16. Chen K, Piknova B, Pittman RN, Schechter AN, and Popel AS. Nitric oxide from nitrite reduction by hemoglobin in the plasma and erythrocytes. *Nitric Oxide* 18: 47–60, 2008.
 17. Chen K and Popel AS. Theoretical analysis of biochemical pathways of nitric oxide release from vascular endothelial cells. *Free Radic Biol Med* 41: 668–680, 2006.
 18. Chen K and Popel AS. Vascular and perivascular NO release and transport: biochemical pathways of NOS1 and NOS3. *Free Radic Biol Med* 42: 811–822, 2007.
 19. Chen K, Popel AS, and Pittman RN. Vascular smooth muscle no exposure from intraerythrocytic SNOHb: a mathematical model. *Antioxid Redox Signal* 9: 1097–1110, 2007.
 20. Chen X, Buerk DG, Barbee KA, and Jaron D. A model of NO/O₂ transport in capillary-perfused tissue containing an arteriole and venule pair. *Ann Biomed Eng* 35: 517–529, 2007.
 21. Chen X, Jaron D, Barbee KA, and Buerk DG. The influence of radial RBC distribution, blood velocity profiles, and glycocalyx on coupled NO/O₂ transport. *J Appl Physiol* 100: 482–492, 2006.
 22. Chu A, Chambers DE, Lin CC, Kuehl WD, Palmer RM, Moncada S, and Cobb FR. Effects of inhibition of nitric oxide formation on basal vasomotion and endothelium-dependent responses of the coronary arteries in awake dogs. *J Clin Invest* 87: 1964–1968, 1991.
 23. Condorelli P and George SC. In vivo control of soluble guanylate cyclase activation by nitric oxide: a kinetic analysis. *Biophys J* 80: 2110–2119, 2001.
 24. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO 3rd, and Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med* 9: 1498–1505, 2003.
 25. Cristina de Assis M, Cristina Plotkowski M, Fierro IM, Barja-Fidalgo C, and de Freitas MS. Expression of inducible nitric oxide synthase in human umbilical vein endothelial cells during primary culture. *Nitric Oxide* 7: 254–261, 2002.
 26. Daiber A, Shoun H, and Ullrich V. Nitric oxide reductase (P450nor) from *Fusarium oxysporum*. *J Inorg Biochem* 99: 185–193, 2005.
 27. Dalsgaard T, Simonsen U, and Fago A. Nitrite-dependent vasodilation is facilitated by hypoxia and is independent of known NO-generating nitrite reductase activities. *Am J Physiol Heart Circ Physiol* 292: H3072–H3078, 2007.
 28. Dickson A, Lin J, Sun J, Broderick M, Harry FA, and Zhang XJ. Construction and characterization of a new flexible and nonbreakable nitric oxide microsensor. *Electroanalysis* 16: 640–643, 2004.
 29. El-Farra NH, Christofides PD, and Liao JC. Analysis of nitric oxide consumption by erythrocytes in blood vessels using a distributed multicellular model. *Ann Biomed Eng* 31: 294–309, 2003.
 30. Elayan IM, Axley MJ, Prasad PV, Ahlers ST, and Auker CR. Effect of hyperbaric oxygen treatment on nitric oxide and oxygen free radicals in rat brain. *J Neurophysiol* 83: 2022–2029, 2000.
 31. Ellsworth ML, Forrester T, Ellis CG, and Dietrich HH. The erythrocyte as a regulator of vascular tone. *Am J Physiol* 269: H2155–H2161, 1995.
 32. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnette D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ, Loscalzo J, and Stamler JS. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci U S A* 90: 10957–10961, 1993.
 33. Gautier C, van Faassen E, Mikula I, Martasek P, and Slama-Schwok A. Endothelial nitric oxide synthase reduces nitrite anions to NO under anoxia. *Biochem Biophys Res Commun* 341: 816–821, 2006.
 34. Golub AS, Barker MC, and Pittman RN. PO₂ profiles near arterioles and tissue oxygen consumption in rat mesentery. *Am J Physiol Heart Circ Physiol* 293: H1097–H1106, 2007.
 35. Haas TL and Duling BR. Morphology favors an endothelial cell pathway for longitudinal conduction within arterioles. *Microvasc Res* 53: 113–120, 1997.
 36. Hilarius PM, Goedhart PT, Ince C, and Verhoeven AJ. Human erythrocytes contain negligible amounts of endothelial type NO synthase (eNOS): Abstracts of the Second International Meeting of the Role of Nitrite in Physiology. *Pathophysiol Therapeut* 90–91, 2007.
 37. Hood JD, Meininger CJ, Ziche M, and Granger HJ. VEGF up-regulates eNOS message, protein, and NO production in human endothelial cells. *Am J Physiol* 274: H1054–1058, 1998.
 38. Huang A, Sun D, Shesely EG, Levee EM, Koller A, and Kaley G. Neuronal NOS-dependent dilation to flow in coronary arteries of male eNOS-KO mice. *Am J Physiol Heart Circ Physiol* 282: H429–H436, 2002.
 39. Huang Z, Shiva S, Kim-Shapiro DB, Patel RP, Ringwood LA, Irby CE, Huang KT, Ho C, Hogg N, Schechter AN, and Gladwin MT. Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J Clin Invest* 115: 2099–2107, 2005.
 40. Hunter CJ, Dejam A, Blood AB, Shields H, Kim-Shapiro DB, Machado RF, Tarekegn S, Mulla N, Hopper AO, Schechter AN, Power GG, and Gladwin MT. Inhaled nebulized nitrite is a hypoxia-sensitive NO-dependent selective pulmonary vasodilator. *Nat Med* 10: 1122–1127, 2004.
 41. Iwakiri Y, Satoh A, Chatterjee S, Toomre DK, Chalouni CM, Fulton D, Groszmann RJ, Shah VH, and Sessa WC. Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. *Proc Natl Acad Sci U S A* 103: 19777–19782, 2006.
 42. Jeffers A, Gladwin MT, and Kim-Shapiro DB. Computation of plasma hemoglobin nitric oxide scavenging in hemolytic anemias. *Free Radic Biol Med* 41: 1557–1565, 2006.
 43. Jeffers A, Xu X, Huang KT, Cho M, Hogg N, Patel RP, and Kim-Shapiro DB. Hemoglobin mediated nitrite activation of soluble guanylyl cyclase. *Comp Biochem Physiol A Mol Integr Physiol* 142: 130–135, 2005.
 44. Joshi MS, Ferguson TB Jr, Han TH, Hyde DR, Liao JC, Ras-saf T, Bryan N, Feelisch M, and Lancaster JR Jr. Nitric oxide is consumed, rather than conserved, by reaction with oxyhemoglobin under physiological conditions. *Proc Natl Acad Sci U S A* 99: 10341–10346, 2002.
 45. Kashiwagi S, Kajimura M, Yoshimura Y, and Suematsu M. Nonendothelial source of nitric oxide in arterioles but not in venules: alternative source revealed in vivo by diamino-fluorescein microfluorography. *Circ Res* 91: e55–e64, 2002.
 46. Kavdia M and Popel AS. Contribution of nNOS- and eNOS-derived NO to microvascular smooth muscle NO exposure. *J Appl Physiol* 97: 293–301, 2004.

47. Kavdia M and Popel AS. Venular endothelium-derived NO can affect paired arteriole: a computational model. *Am J Physiol Heart Circ Physiol* 290: H716–H723, 2006.
48. Kavdia M and Popel AS. Wall shear stress differentially affects NO level in arterioles for volume expanders and Hb-based O₂ carriers. *Microvasc Res* 66: 49–58, 2003.
49. Kimura C, Oike M, Ohnaka K, Nose Y, and Ito Y. Constitutive nitric oxide production in bovine aortic and brain microvascular endothelial cells: a comparative study. *J Physiol* 554: 721–730, 2004.
50. Kjellstrom BT, Ortenwall P, and Risberg B. Comparison of oxidative metabolism in vitro in endothelial cells from different species and vessels. *J Cell Physiol* 132: 578–580, 1987.
51. Kleinbongard P, Schulz R, Rassaf T, Lauer T, Dejam A, Jax T, Kumara I, Gharini P, Kabanova S, Ozuyaman B, Schnurch HG, Godecke A, Weber AA, Robenek M, Robenek H, Bloch W, Rosen P, and Kelm M. Red blood cells express a functional endothelial nitric oxide synthase. *Blood* 107: 2943–2951, 2006.
52. Koivisto A, Mathias A, Bronnikov G, and Nedergaard J. Kinetics of the inhibition of mitochondrial respiration by NO. *FEBS Lett* 417: 75–80, 1997.
53. Kollau A, Hofer A, Russwurm M, Koesling D, Keung WM, Schmidt K, Brunner F, and Mayer B. Contribution of aldehyde dehydrogenase to mitochondrial bioactivation of nitroglycerin: evidence for the activation of purified soluble guanylate cyclase through direct formation of nitric oxide. *Biochem J* 385: 769–777, 2005.
54. Kuchan MJ and Frangos JA. Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *Am J Physiol* 266: C628–C636, 1994.
55. Lacza Z, Pankotai E, Csordas A, Gero D, Kiss L, Horvath EM, Kollai M, Busija DW, and Szabo C. Mitochondrial NO and reactive nitrogen species production: does mtNOS exist? *Nitric Oxide* 14: 162–168, 2006.
56. Lamkin-Kennard KA, Buerk DG, and Jaron D. Interactions between NO and O₂ in the microcirculation: a mathematical analysis. *Microvasc Res* 68: 38–50, 2004.
57. Lancaster JR Jr. Simulation of the diffusion and reaction of endogenously produced nitric oxide. *Proc Natl Acad Sci U S A* 91: 8137–8141, 1994.
58. Landar A and Darley-Usmar VM. Evidence for oxygen as the master regulator of the responsiveness of soluble guanylate cyclase and cytochrome c oxidase to nitric oxide. *Biochem J* 405: e3–e4, 2007.
59. Li H, Liu X, Cui H, Chen YR, Cardounel AJ, and Zweier JL. Characterization of the mechanism of cytochrome P450 reductase-cytochrome P450-mediated nitric oxide and nitrosothiol generation from organic nitrates. *J Biol Chem* 281: 12546–12554, 2006.
60. Li H, Samouilov A, Liu X, and Zweier JL. Characterization of the magnitude and kinetics of xanthine oxidase-catalyzed nitrite reduction: evaluation of its role in nitric oxide generation in anoxic tissues. *J Biol Chem* 276: 24482–24489, 2001.
61. Liu X, Yan Q, Baskerville KL, and Zweier JL. Estimation of nitric oxide concentration in blood for different rates of generation: evidence that intravascular nitric oxide levels are too low to exert physiological effects. *J Biol Chem* 282: 8831–8836, 2007.
62. Lu P, Liu F, Yao Z, Wang CY, Chen DD, Tian Y, Zhang JH, and Wu YH. Nitrite-derived nitric oxide by xanthine oxidoreductase protects the liver against ischemia-reperfusion injury. *Hepatology* 41: 350–355, 2005.
63. Lubrano V, Vassalle C, Blandizzi C, Del Tacca M, Palombo C, L'Abbate A, Baldi S, and Natali A. The effect of lipoproteins on endothelial nitric oxide synthase is modulated by lipoperoxides. *Eur J Clin Invest* 33: 117–125, 2003.
64. Luchsinger BP, Rich EN, Gow AJ, Williams EM, Stamler JS, and Singel DJ. Routes to S-nitroso-hemoglobin formation with heme redox and preferential reactivity in the beta subunits. *Proc Natl Acad Sci U S A* 100: 461–466, 2003.
65. Malinski T, Taha Z, Grunfeld S, Patton S, Kaptureczak M, and Tomboulian P. Diffusion of nitric oxide in the aorta wall monitored in situ by porphyrinic microsensors. *Biochem Biophys Res Commun* 193: 1076–1082, 1993.
66. Mansuy D and Boucher JL. Alternative nitric oxide-producing substrates for NO synthases. *Free Radic Biol Med* 37: 1105–1121, 2004.
67. Moller MN, Li Q, Vitturi DA, Robinson JM, Lancaster JR Jr, and Denicola A. Membrane “lens” effect: focusing the formation of reactive nitrogen oxides from the *NO/O₂ reaction. *Chem Res Toxicol* 20: 709–714, 2007.
68. Moncada S and Higgs EA. Nitric oxide and the vascular endothelium. *Handb Exp Pharmacol* 176 Pt1: 213–254, 2006.
69. Nagano T and Yoshimura T. Bioimaging of nitric oxide. *Chem Rev* 102: 1235–1270, 2002.
70. Nalwaya N and Deen WM. Analysis of the effects of nitric oxide and oxygen on nitric oxide production by macrophages. *J Theoret Biol* 226: 409–419, 2004.
71. Nase GP, Tuttle J, and Bohlen HG. Reduced perivascular PO₂ increases nitric oxide release from endothelial cells. *Am J Physiol Heart Circ Physiol* 285: H507–H515, 2003.
72. Nolte D, Botzlar A, Pickelmann S, Bouskela E, and Messmer K. Effects of diaspirin-cross-linked hemoglobin (DCLHb) on the microcirculation of striated skin muscle in the hamster: a study on safety and toxicity. *J Lab Clin Med* 130: 314–327, 1997.
73. Ongini E, Impagnatiello F, Bonazzi A, Guzzetta M, Govoni M, Monopoli A, Del Soldato P, and Ignarro LJ. Nitric oxide (NO)-releasing statin derivatives, a class of drugs showing enhanced antiproliferative and antiinflammatory properties. *Proc Natl Acad Sci U S A* 101: 8497–8502, 2004.
74. Pacher P, Beckman JS, and Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87: 315–424, 2007.
75. Panza JA, Casino PR, Kilcoyne CM, and Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation* 87: 1468–1474, 1993.
76. Pittman RN. Oxygen transport and exchange in the microcirculation. *Microcirculation* 12: 59–70, 2005.
77. Qiu Y, Sutton L, and Riggs AF. Identification of myoglobin in human smooth muscle. *J Biol Chem* 273: 23426–23432, 1998.
78. Rengasamy A and Johns RA. Determination of K_m for oxygen of nitric oxide synthase isoforms. *J Pharmacol Exp Ther* 276: 30–33, 1996.
79. Robinson JK, Bollinger MJ, and Birks JW. Luminol/H₂O₂ chemiluminescence detector for the analysis of nitric oxide in exhaled breath. *Analyt Chem* 71: 5131–5136, 1999.
80. Robinson JM and Lancaster JR Jr. Hemoglobin-mediated, hypoxia-induced vasodilation via nitric oxide: mechanism(s) and physiologic versus pathophysiologic relevance. *Am J Respir Cell Mol Biol* 32: 257–261, 2005.
81. Rodriguez-Juarez F, Aguirre E, and Cadenas S. Relative sensitivity of soluble guanylate cyclase and mitochondrial respiration to endogenous nitric oxide at physiological oxygen concentration. *Biochem J* 405: 223–231, 2007.
82. Roy B and Garthwaite J. Nitric oxide activation of guanylyl cyclase in cells revisited. *Proc Natl Acad Sci U S A* 103: 12185–12190, 2006.
83. Russwurm M, Behrends S, Harteneck C, and Koesling D. Functional properties of a naturally occurring isoform of soluble guanylyl cyclase. *Biochem J* 335: 125–130, 1998.
84. Santhanam L, Lim HK, Lim HK, Miriel V, Brown T, Patel M, Balanson S, Ryoo S, Anderson M, Irani K, Khanday F, Di Costanzo L, Nyhan D, Hare JM, Christianson DW, Rivers R, Shoukas A, and Berkowitz DE. Inducible NO synthase dependent S-nitrosylation and activation of arginase 1 contribute to age-related endothelial dysfunction. *Circ Res* 101: 692–702, 2007.
85. Santolini J, Meade AL, and Stuehr DJ. Differences in three kinetic parameters underpin the unique catalytic profiles of nitric-oxide synthases I, II, and III. *J Biol Chem* 276: 48887–48898, 2001.
86. Schechter AN and Gladwin MT. Hemoglobin and the paracrine and endocrine functions of nitric oxide. *N Engl J Med* 348: 1483–1485, 2003.
87. Shibata M, Ichioka S, and Kamiya A. Nitric oxide modulates oxygen consumption by arteriolar walls in rat skeletal muscle. *Am J Physiol Heart Circ Physiol* 289: H2673–H2679, 2005.

88. Shiva S, Huang Z, Grubina R, Sun J, Ringwood LA, MacArthur PH, Xu X, Murphy E, Darley-Usmar VM, and Gladwin MT. Deoxyglobin is a nitrite reductase that generates nitric oxide and regulates mitochondrial respiration. *Circ Res* 100: 654–661, 2007.
89. Sibmoo N, Piknova B, Rizzatti F, and Schechter AN. Nitrite generation in human erythrocytes from NO derivatives of hemoglobin by dehydroascorbic acid. *Abstracts of Second International Meeting on the Role of Nitrite in Physiology. Pathophysiol Therapeut* 69, 2007.
90. Silva HS, Kapela A, and Tsoukias NM. A mathematical model of plasma membrane electrophysiology and calcium dynamics in vascular endothelial cells. *Am J Physiol Cell Physiol* 293: C277–C293, 2007.
91. Singel DJ and Stamler JS. Blood traffic control. *Nature* 430: 297, 2004.
92. Singel DJ and Stamler JS. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. *Annu Rev Physiol* 67: 99–145, 2005.
93. Sonveaux P, Lobysheva, II, Feron O, and McMahon TJ. Transport and peripheral bioactivities of nitrogen oxides carried by red blood cell hemoglobin: role in oxygen delivery. *Physiology (Bethesda)* 22: 97–112, 2007.
94. Sprague RS, Ellsworth ML, Stephenson AH, and Lonigro AJ. Participation of cAMP in a signal-transduction pathway relating erythrocyte deformation to ATP release. *Am J Physiol Cell Physiol* 281: C1158–C1164, 2001.
95. Stone JR and Marletta MA. Spectral and kinetic studies on the activation of soluble guanylate cyclase by nitric oxide. *Biochemistry* 35: 1093–1099, 1996.
96. Stuehr DJ, Santolini J, Wang ZQ, Wei CC, and Adak S. Update on mechanism and catalytic regulation in the NO synthases. *J Biol Chem* 279: 36167–36170, 2004.
97. Suematsu M, Sukanuma K, and Kashiwagi S. Mechanistic probing of gaseous signal transduction in microcirculation. *Antioxid Redox Signal* 5: 485–492, 2003.
98. Talukder MA, Fujiki T, Morikawa K, Motoishi M, Kubota H, Morishita T, Tsutsui M, Takeshita A, and Shimokawa H. Up-regulated neuronal nitric oxide synthase compensates coronary flow response to bradykinin in endothelial nitric oxide synthase-deficient mice. *J Cardiovasc Pharmacol* 44: 437–445, 2004.
99. Thomas DD, Liu X, Kantrow SP, and Lancaster JR Jr. The biological lifetime of nitric oxide: implications for the perivascular dynamics of NO and O₂. *Proc Natl Acad Sci U S A* 98: 355–360, 2001.
100. Tsai AG, Acero C, Nance PR, Cabrales P, Frangos JA, Buerk DG, and Intaglietta M. Elevated plasma viscosity in extreme hemodilution increases perivascular nitric oxide concentration and microvascular perfusion. *Am J Physiol Heart Circ Physiol* 288: H1730–H1739, 2005.
101. Tsai AG, Cabrales P, Manjula BN, Acharya SA, Winslow RM, and Intaglietta M. Dissociation of local nitric oxide concentration and vasoconstriction in the presence of cell-free hemoglobin oxygen carriers. *Blood* 108: 3603–3610, 2006.
102. Tsai AG, Friesenecker B, Mazzoni MC, Kerger H, Buerk DG, Johnson PC, and Intaglietta M. Microvascular and tissue oxygen gradients in the rat mesentery. *Proc Natl Acad Sci U S A* 95: 6590–6595, 1998.
103. Tsikas D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: appraisal of the Griess reaction in the L-arginine/nitric oxide area of research. *J Chromatogr B Anal Technol Biomed Life Sci* 851: 51–70, 2007.
104. Tsoukias NM, Kavdia M, and Popel AS. A theoretical model of nitric oxide transport in arterioles: frequency- vs. amplitude-dependent control of cGMP formation. *Am J Physiol Heart Circ Physiol* 286: H1043–H1056, 2004.
105. Tsoukias NM and Popel AS. A model of nitric oxide capillary exchange. *Microcirculation* 10: 479–495, 2003.
106. Vadapalli A, Pittman RN, and Popel AS. Estimating oxygen transport resistance of the microvascular wall. *Am J Physiol Heart Circ Physiol* 279: H657–H671, 2000.
107. Vaughn MW, Kuo L, and Liao JC. Estimation of nitric oxide production and reaction rates in tissue by use of a mathematical model. *Am J Physiol* 274: H2163–H2176, 1998.
108. Vukosavljevic N, Jaron D, Barbee KA, and Buerk DG. Quantifying the L-arginine paradox in vivo. *Microvasc Res* 71: 48–54, 2006.
109. Webb AJ, Rathod KS, Lovell MJ, Chu WL, Lecomte FM, Uppal R, Raimondo C, Khoshbin E, Ahmed A, Hobbs AJ, and Ahluwalia A. Nitrite is reduced to NO by xanthine oxidoreductase and NO synthase in the erythrocyte membrane during hypoxemia. *Abstracts of Second International Meeting on the Role of Nitrite in Physiology, Pathophysiol Therapeut* 134–135, 2007.
110. Wennmalm A, Lanne B, and Petersson AS. Detection of endothelial-derived relaxing factor in human plasma in the basal state and following ischemia using electron paramagnetic resonance spectrometry. *Anal Biochem* 187: 359–363, 1990.
111. Wilson DF, Lee WM, Makonnen S, Finikova O, Apreleva S, and Vinogradov SA. Oxygen pressures in the interstitial space and their relationship to those in the blood plasma in resting skeletal muscle. *J Appl Physiol* 101: 1648–1656, 2006.
112. Yan Q, Liu Q, Zweier JL, and Liu X. Potency of authentic nitric oxide in inducing aortic relaxation. *Pharmacol Res* 55: 329–334, 2007.
113. Zani BG and Bohlen HG. Transport of extracellular l-arginine via cationic amino acid transporter is required during in vivo endothelial nitric oxide production. *Am J Physiol Heart Circ Physiol* 289: H1381–H1390, 2005.
114. Zhang X, Kim WS, Hatcher N, Potgieter K, Moroz LL, Gillette R, and Sweedler JV. Interfering with nitric oxide measurements: 4,5-diaminofluorescein reacts with dehydroascorbic acid and ascorbic acid. *J Biol Chem* 277: 48472–48478, 2002.
115. Zhao Y, Brandish PE, Ballou DP, and Marletta MA. A molecular basis for nitric oxide sensing by soluble guanylate cyclase. *Proc Natl Acad Sci U S A* 96: 14753–14758, 1999.
116. Zhou XS, Bohlen HG, Miller SJ, and Unthank JL. Direct measurement of periarterial nitric oxide in SHR reveals elevated basal levels and suppressed flow-mediated production. *Abstracts of Eighth World Congress for Microcirculation* 530–531, 2007.
117. Zweier JL, Samouilov A, and Kuppusamy P. Non-enzymatic nitric oxide synthesis in biological systems. *Biochim Biophys Acta* 1411: 250–262, 1999.

Address reprint requests to:

Kejing Chen, Ph.D.

Department of Biomedical Engineering
Johns Hopkins University School of Medicine
613 Traylor Building, 720 Rutland Avenue
Baltimore, MD 21205

E-mail: kchen21@jhu.edu

Date of first submission to ARS Central, October 18, 2007; date of final revised submission, November 27, 2007; date of acceptance, December 16, 2007.

This article has been cited by:

1. Sunil J Wimalawansa. 2008. Nitric oxide: new evidence for novel therapeutic indications. *Expert Opinion on Pharmacotherapy* 9:11, 1935-1954. [[CrossRef](#)]