Nitrite as a vascular endocrine nitric oxide reservoir that contributes to hypoxic signaling, cytoprotection, and vasodilation

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Hypoxic vasodilation is a conserved systemic physiological response that matches blood flow and oxygen delivery to tissue metabolic demand. This hypoxic response has been appreciated for more than 100 years since the initial description by Roy and Brown in 1879 (80). This response is thought to involve feedback mechanisms that require oxygen or pH sensing of a divergence in the normal relationship between delivered blood oxygen and tissue oxygen consumption (94). This leads to the feedback generation of putative vasodilatory effectors that increase blood flow to maintain adequate tissue oxygenation. Important to the considerations of the mechanisms responsible for oxygen sensing, in mammals hypoxic vasodilation appears to occur as the hemoglobin desaturates from 60% to 40%, around a partial pressure of oxygen ranging from 40–20 mmHg (79).

Surprisingly, measurements of microcirculatory and tissue oxygen tension and hemoglobin oxygen saturation using modern methodologies suggest that much of the oxygen delivery occurs within the resistance arterioles, especially in the case of skeletal muscle (91). Thus, in these microvascular beds, the anatomical site of hypoxic sensing is proximal to the site of resistive control (arterioles and arteriolar capillaries). In other tissues, such as heart and brain, more oxygen is extracted within the capillary network. This creates a paradox as to how hypoxic sensing can modulate retrograde feedback vasodilation in these tissues. The solution to this paradox has been in part solved by the work of Segal and Duling (84–86), who suggested that acetylcholine-dependent vasodilation of the capillary or venous circulation produces retrograde intracellular propagation of a vasodilating signal to the precapillary resistance vessels. Additional recent hypotheses suggest diffusional shunting of nitric oxide (NO) from veins to an adjacent arteriole; several organs have a circulation where there are two adjacent venules next to arterioles, thus potentially allowing for this effect (52). These data in aggregate suggest that the mechanisms responsible for oxygen sensing are responsive to tissue oxygen partial pressures of 20–40 mmHg and hemoglobin saturations of 40–60% (around the hemoglobin P50). The site of oxygen sensing and vasodilation occurs either within the A3-A4 arterioles (and muscularized capillaries) or in the capillary network, with the latter associated with retrograde propagation of a vasodilating signal through endothelium to the precapillary resistance arterioles.

Despite the physiological appreciation of the hypoxic vasodilation response, the identities of the oxygen sensor mechanism and the specific feedback vasodilator effectors remain
uncertain. Although a number of mediators have been considered, the specific blockade of many of these pathways fails to completely inhibit hypoxic vasodilation (94). The mediators that have been considered include adenosine, NO, ATP-sensitive \( \mathrm{K}^+ \) channels, endothelium-derived hyperpolarizing factor (candidates include \( \mathrm{CO}, \mathrm{H}_2\mathrm{O}_2 \), or \( \mathrm{ONO}^- \)), and prostacyclin (94, 95). These observations indicate the presence of multiple overlapping and integrated mechanisms, or that other undiscovered pathways exist, and serve to highlight the critical physiological importance of hypoxic vasodilation.

**ROLE FOR RED BLOOD CELL AND HEMOGLOBIN IN HYPOXIC VASODILATION**

A new paradigm for hypoxic vasodilation was advanced in 1995 that suggested that hemoglobin per se is the oxygen sensor with the oxygen-linked allosteric structural transition of the hemoglobin tetramer from the oxygenated conformation (relaxed or R state) to the deoxygenated conformation (tense or T state), signaling the release or generation of a vasodilating signal from the erythrocyte (19). The first such hypothesis suggested that this R-to-T transition was coupled to the release of ATP from the erythrocyte, which by binding to purinergic receptors in endothelium resulted in vasodilation (16, 19, 32, 48). This mechanism is supported by the observations of increasing concentrations of ATP in venous blood after hypoxia or physiological acidemia, the in vitro release of ATP by hypoxic or acidic erythrocytes, and the retrograde propagation of vasodilation from the capillaries to precapillary arterioles after ATP/purinergic receptor/endothelial NO synthase signaling.

The second hypotheses suggests that hemoglobin deoxygenation results in NO (equivalent) release from the red blood cell and subsequent NO-dependent vasodilation: two fundamentally different mechanisms of red blood cell-mediated NO release have been proposed. The first proposed mechanism is that \( S \)-nitrosated hemoglobin (SNO-Hb) releases \( S \)-nitrosothiols during hemoglobin deoxygenation with subsequent vasodilation (33, 34, 50, 88). The second proposed mechanism suggests that hemoglobin is an allosterically regulated heme-based nitrite reductase that reduces nitrite to NO as hemoglobin deoxygénantes (10, 39, 41, 66).

Whereas we support the principle advanced by Stamler and colleagues (50, 88) that the red blood cell transduces hypoxic NO bioactivity, their proposed mechanisms have been challenged by multiple laboratories, and the reader is encouraged to read these studies and formulate an independent assessment (6, 12–15, 25–27, 30, 37, 38, 40, 42, 51, 73, 77, 99, 100). In this review, the evidence supporting the nitrite reductase mechanism and the global role of nitrite as an endocrine NO reservoir and intrinsic signaling molecule will be detailed.

**ENDOCRINE PROPERTIES OF NO?**

NO is produced from endothelial NO synthase and participates in the regulation of basal blood vessel tone and vascular homeostasis (antithromplatelet activity, modulation of oxidative/nitrosative stress and inflammation, endothelial and smooth muscle proliferation, and adhesion molecule expression) (22, 44, 45, 71, 72). NO is a paracrine signaling molecule because it is produced in endothelium and then diffuses to vicinal smooth muscle, binds avidly to the heme of soluble guanylyl cyclase, which produces cGMP, activates cGMP-dependent protein kinases, and ultimately produces smooth muscle relaxation.

NO that diffuses into the lumen of the blood vessel is expected to react at a nearly diffusion-limited rate (\( 10^7 \mathrm{M}^{-1} \mathrm{s}^{-1} \)) with both oxy- and deoxyhemoglobin to form methemoglobin/nitrate and iron-nitrosyl-hemoglobin (HbFe\(^{II} \)), respectively (26, 70). While this reaction is reduced by hemoglobin compartmentalization within the erythrocyte, these reactions still greatly limit the half-life and diffusional distance of NO in blood (<2 ms half-life in blood and <2 \( \mu \)s in lysed blood) and largely maintain NO as a paracrine vaso-regulator (1, 54). Accordingly, the inhalation of NO gas produces selective pulmonary vasodilation and no appreciable changes in systemic blood pressure. However, a growing number of studies suggest a subtle but measurable systemic effect of inhaled NO on systemic perfusion, especially with a higher dose NO gas inhalation (80 parts/million (ppm)) and concomitant inhibition of peripheral endothelial NO production. In these studies, inhaled NO has been shown to 1) increase urinary flow in pigs (90), 2) decrease systemic vascular resistance in septic dogs (75), 3) decrease systemic vascular resistance in anesthetized sheep (89), 4) increase intestinal blood flow during concurrent NO synthase inhibition or after intestinal ischemia-reperfusion injury in cats (20, 56, 68), 5) prevent forearm vasoconstriction during regional NO synthase inhibition in human volunteers (9, 101, 102), 6) increase contralateral forearm blood flow after infusion of NO solution into ipsilateral forearm in human volunteers (78), and 7) decrease the size of myocardial infarction in mice (36).

From a biochemical standpoint, there is increasing appreciation that NO may be stabilized in blood by the formation of NO-modified proteins, peptides and lipids, as well as by oxidation to the anion nitrite. The principle that NO may be thus stabilized in blood, and the inactivation reactions with hemoglobin thus limited, was first proposed by Loscalzo and colleagues. They hypothesized that NO, upon abstraction of an electron, could form a covalent bond with cysteine residues on albumin to form \( S \)-nitrosated albumin (SNO-albumin) (81, 87). This paradigm was later extended by Stamler’s laboratory (50) to SNO-Hb (50). It is likely that there are a number of intravascular species capable of endocrine vasodilation, including \( S \)-nitrosothiols (68, 87), nitrite (10, 29, 64, 92), \( N \)-nitrosamines (35, 59, 76, 96), iron-nitrosyls (26), and the recently identified nitrated lipids (2, 58, 82, 83). Accumulating data from our laboratory and others suggest that nitrite may be a major stable reservoir of NO in the circulation and that this molecule may singularly account for the observed endocrine manifestations of NO gas inhalation.

**VASOACTIVITY OF NITRITE IN HUMAN CIRCULATION**

While large doses of nitrite given as an antidote for cyanide poisoning clearly produce hypotension in humans (98), the large concentrations of nitrite required to vasodilate aortic ring bioassay systems, at room oxygen and neutral pH, led to a dismissal of nitrite as a physiological vasoactive mediator. Indeed, nitrite at concentrations of 100 \( \mu \)M was shown to vasodilate aortic ring bioassays by Furchgott as far back as 1953 and shown by Murad and Ignarro to activate guanylate cyclase in the mid-1970s and early 1980s (21, 46, 47, 63).
However, studies published by Lauer and colleagues demonstrated that nitrite had no vasodilator activity when infused at concentrations of 200 μM in the forearm of three normal volunteers. This observation appeared to close the door on the notion that nitrite was a physiological vasodilator (57, 61, 74).

Despite the apparent lack of bioactivity of nitrite in these more recent studies (29), we observed artery-to-vein gradients in nitrite across the human forearm, with increased consumption of nitrite during exercise stress, suggesting that nitrite was metabolized across the peripheral circulation. Furthermore, when humans were exposed to 80 ppm inhaled NO gas, we observed an increase in peripheral forearm blood flow that was only associated with increases in plasma nitrite; we observed no significant increase in plasma SNO-albumin or erythrocyte SNO-Hb (9). We considered the possibility that nitrite might be reduced to NO during physiological hypoxic and acidic stress by the actions of xanthine oxidoreductase (31, 62) or by acidic reduction (disproportionation) (60, 64, 103). To test this hypothesis, we infused nitrite into the forearm brachial artery of 28 healthy volunteers and, to our surprise, observed substantial vasodilation, even without exercise stress. Nitrite was remarkably potent, increasing blood flow by 170% at 200 μM and by 22% at 2.5 μM. Even levels of 900 nM produced vasodilation during exercise stress with concurrent NO synthase inhibition with Nω-monomethyl-L-arginine (l-NMMA) (Fig. 1) (10). Additional studies (43, 55, 92, 93, 97) have recently been published confirming the potent vasodilating effects of nitrite. Our group and others have now observed vasodilation at near physiological concentrations (<5 μM) in mice, rats, sheep, dogs, primates, and humans.

**EVIDENCE THAT NITRITE IS ENDOCRINE NO SPECIES PRODUCING PERIPHERAL EFFECTS DURING NO GAS INHALATION**

Perhaps the strongest data supporting the thesis that nitrite is the major endocrine NO species in blood come from studies of ischemia-reperfusion over the last two years. These studies consistently show that levels of nitrite, just above the physiological, potently inhibit ischemia-reperfusion apoptotic cell...
death. Webb and colleagues (97) published studies showing that levels of nitrite as low as 10 μM potently inhibited ischemia-reperfusion injury in the rat Langendorff heart model. Duranski and colleagues (18) reported that nitrite limited ischemia-reperfusion cytotoxicity at doses as low as 1.2 nmol and increases in blood levels as low as 200 nM. In fact, the myocardial infarction relative to the area at risk was decreased by 50% with increases in plasma nitrite from the basal level of 700 nM to only 900 nM. Studies by Ng and colleagues (68), evaluating the effects of 80 ppm NO gas inhalation on feline intestinal ischemia-reperfusion blood flow, revealed increases in plasma nitrite from ∼100 to 489 nM (68), well above the effective doses of nitrite observed in the Duranski studies. These increases from the basal level of 100 to 489 nM were associated with protection from ischemia-reperfusion impairments in microvascular perfusion. Similarly, in a recent study by Hataishi and colleagues (36), inhalation of 80 ppm NO gas for 20 min in mice was associated with a 800 nM increase in plasma nitrite (4.5-fold increase) and a 660 nM increase in whole blood nitrite (plasma and red blood cell), with no increase in plasma S-nitrosothiols; this was associated with a 50% decrease in myocardial infarction area. Finally, in a recent study presented at the National Institutes of Health (NIH) Nitrite Meeting by Andrew Arai, from the NIH Laboratory of Cardiac Energetics, a 5-min infusion of nitrite increased plasma levels of nitrite in dogs from a basal level of ∼1 to 5 μM with no associated increases in plasma or red blood cell S-nitrosothiol. These near-physiological increases in nitrite decreased myocardial infarction size from 70% to 20% of the area at risk (28). In aggregate, these data strongly support the thesis that nitrite is the endocrine NO species accounting for the systemic effects of NO gas inhalation. This thesis should be relatively simple to experimentally validate by the addition of a nitrite-infusion control to experiments of 80 ppm NO gas inhalation in these various models.

It should be noted that the administration of nitrite failed to ameliorate ischemia-reperfusion injury in the rat kidney, suggesting organ-specific effects (3). However, the chronic consumption of nitrite in drinking water decreased blood pressure in the spontaneously hypertensive rat (4, 93) and also inhibited renal injury associated with NO synthase inhibition with L-NMMA (69).

GLOBAL ROLE FOR NITRITE IN HYPOXIC SIGNALING

The potent effect of extremely low doses of nitrite on limiting ischemia-reperfusion infarction suggests that nitrite may modulate physiological stress responses, particularly those characterized by tissue ischemia (28). Indeed, the lowest doses given to mice to inhibit ischemia-reperfusion injury, 1.2 nmol, are less than the levels achieved in the circulation after eating a spinach salad (5). Consistent with a role for nitrite in hypoxic signaling, we have observed effects of nitrite on liver and aortic ring soluble guanylate cyclase-dependent signaling (12, 18), modulation of mitochondrial respiration (12, 23), and response to ischemia-reperfusion (18). Bryan and colleagues (8) observed that changes in dietary nitrite modulated stress response pathways, such as heat shock protein 70 and heme-oxygenase 1 expression, in tissues. A role for nitrite as an intrinsic signaling molecule suggests two evolved pathways in NO homeostasis: the oxygen and L-arginine-dependent NO synthase pathway and the hypoxia-dependent nitrite reductase pathway (23).

HEMOGLOBIN AS ALLOSTERICALLY AND REDOX-REGULATED NITRITE REDUCTASE

Vasodilation mediated by near-physiological concentrations of nitrite under normal physiological nonstress conditions appears to be inconsistent with a mechanism of nitrite reduction by xanthine oxidoreductase or disproportionation, because both of these pathways require very low pH and near anoxia. Because xanthine oxidoreductase also produces superoxide (a diffusion-limited NO scavenger) when oxygen is available, the formation of NO from xanthine oxidase can only occur during anoxia or in the presence of high levels of superoxide dismutase. The observation that nitrite infusions produce vasodilation along the physiological oxygen gradient suggests an alternative mechanism of bioactivation.

During nitrite infusions into the brachial artery, we observed the arterial-to-venous formation of HbFeII-NO, suggesting that nitrite was being reduced to NO rapidly within one-half circulatory time (10). An analysis of the HbFeII-NO levels during all experimental conditions (rest, L-NMMA coinfusion, and exercise) revealed a striking inverse correlation with oxyhemoglobin saturation, i.e., as hemoglobin deoxygenated, more NO was formed. These physiological observations were consistent with a reaction between nitrite and deoxyhemoglobin to form NO as described by Brooks in 1937 (7) and by Doyle and colleagues in 1981 (17): NO2− + HbFeIII (deoxygenated hemoglobin) + H+ → NO + HbFeII + OH−.

Much of the formed NO is then captured as HbFeII-NO on vicinal hemes, thus constituting a “dosimeter” of NO production in venous blood: NO + HbFeII → HbFeIII-NO.

We were impressed by the potential physiological implications of this simple equation for hypoxic signaling. The reaction requires deoxyhemoglobin and a proton, providing oxygen and pH sensor chemistry, respectively, and generates NO, a potent vasodilator. Methemoglobin formed during the reaction will not autocapture and inactivate the NO formed within the heme pocket. In additional experiments we found that nitrite, red blood cells (or hemoglobin), and hypoxia were required for in vitro hypoxic vasodilation of rat aortic rings. Indeed, in the presence of hypoxia and erythrocytes (conditions never tested in historical aortic ring bioassay studies), nitrite now vasodilated aortic rings at physiological concentrations of 200–500 nM (10, 12).

Using in vitro aortic ring bioassay systems, designed by the Patel’s laboratory to simultaneously measure vessel force tension and oxygen tension, we found that vasodilation was measurably potentiated by as low as 200 nM nitrite under hypoxic conditions (10, 12). Importantly, these studies revealed that nitrite red blood cell-dependent vasodilation is initiated at an oxygen tension around the hemoglobin P50 (arterial PO2 of 40 mmHg for rat erythrocytes and 30 mmHg for human erythrocytes). Consistent with this, we have observed that this vasodilation occurs as hemoglobin unloads oxygen to ∼50% saturation and that this vasodilation is mediated by a maximal nitrite reductase activity of hemoglobin allosterically linked to its P50 (12, 39, 41).

This maximal reductase activity of hemoglobin is allosterically regulated and peaks around the P50 because of two
opposing chemical factors. The first of these factors involves oxygen binding to hemoglobin that allosterically shifts hemoglobin to the R (relaxed, oxygenated) conformation. R-state hemoglobin exhibits a decreased redox potential of the hemes in the tetramer, making nitrite reduction more thermodynamically favorable, and this correlates with an increase in the nitrite reduction rate (41). Thus R-state hemoglobin has a greater bimolecular rate constant for nitrite reduction (6 M$^{-1}$s$^{-1}$ for R compared with 0.12 M$^{-1}$s$^{-1}$ for T). Please note that this phenomenon is unique for allosteric proteins like hemoglobin. Most reactions are characterized by one bimolecular rate constant, for example the reaction of nitrite with myoglobin has a biomolecular rate constant of 6 M$^{-1}$s$^{-1}$. In the case of hemoglobin, the bimolecular rate constant increases as the hemoglobin undergoes the allosteric structural transition from the T state to the R state because the reaction of nitrite is occurring with a different protein conformation at each stage of this allosteric transition (see Ref. 41 for the measurement of these rate constants at different fractional ligations).

The second chemical factor that leads to a maximal reductase activity around the $P_{50}$ involves the role of the T state or deoxygenated conformation of hemoglobin that has the most nonliganded hemes available for binding and reaction with nitrite (more deoxyheme substrate for nitrite reduction). An ideal balance of available deoxyhemes for nitrite binding, and oxyhemes with a higher bimolecular rate constant for the reaction, is met at the 50% hemoglobin saturation (the $P_{50}$). The rate of a second order reaction is determined by the product of the concentration of two reactants and the bimolecular rate constant. In this case, the nitrite concentration changes only a little as hemoglobin deoxygenates, the deoxyhemoglobin concentration increases dramatically, and the bimolecular rate constant decreases dramatically. So the product of bimo-

**Fig. 2.** Calculated rate of hemoglobin nitrite reduction. Rate of reaction of nitrite with hemoglobin over a full range of hemoglobin ligand states from T state to R state is plotted. Rates were calculated at pH of 7.6 (bottom curve), 7.4 (middle curve), and 7.2 (top curve). These curves do not account for the Bohr effect that (for these changes in pH) would not alter them significantly (41). Rate was calculated as $(R_1)k_R + (4(T_0) + 3(T_1))k_T$, where capital R and T represent quaternary states, subscripts give number of hemes that are ferric or ligand bound (so $R_1$ is R-state Hb with 1 deoxygenated heme), and $k_R$ and $k_T$ are rates for nitrite reaction of each quaternary state. Concentrations of each species (indicated by brackets) was calculated using a MWC model (see Refs. 53 and 65). The value of $c$, the ratio of equilibrium binding constants for T (taken as 1/77 Torr) and R states, was set at 0.015. R-state rate, $k_R$, was set at 60 times $k_T$. Rate of $k_T$ was set to 0.05 for pH 7.6 and varied in direct proportion to concentration of protons as pH was changed.

**Fig. 3.** Nitrite reductase equilibrium along the A1 to A5 arterioles. There exists a steady-state anatomical location within circulation from artery to vein that has the greatest concentration of $R_3$ tetramers (R and T denote oxy- and deoxytetrameric conformation, and number denotes liganded oxygens) that possess the maximal nitrite reductase activity. At this location there would always exist an equilibrium rate constant for nitrite reduction and an equilibrium concentration of nitrite and deoxyhemehmes (maximized in $R_3$ tetramer). Anatomical position of this equilibrium NO concentration will be responsive to tissue metabolism and oxygen consumption by moving the R-to-T transition up- or downstream. In this case, nitrite concentration changes only a little as hemoglobin deoxygenates, as deoxyhemoglobin concentration increases dramatically, and as bimolecular rate constant decreases dramatically as hemoglobin goes from the R-to-T conformation. Hence, product of bimolecular rate constant and deoxyheme concentration peaks from 60–40% hemoglobin oxygen saturation when the most $R_3$ tetramers are present.
lecular rate constant and deoxyheme concentration peaks from 60–40% hemoglobin oxygen saturation. Indeed, the experimentally measured rate of nitrite reduction (and hence NO generation) by hemoglobin is maximal at a hemoglobin-oxygen saturation between 40–60% (41).

The experimental observation of a maximal nitrite reductase rate approximating the P50 is also supported by simulations that show maximum nitrite reductase activity of hemoglobin near the P50 (Fig. 2). In these modeling experiments, the fractional amount of each subspecies (T0, R4, R3, etc.) was calculated using an allosteric model (65), and the rate of nitrite reduction by R-state deoxygenated hemes (as in R3) was assumed to be 60 times faster than for T-state hemes (41). The rates were also calculated at pH values of 7.6, 7.4, and 7.2, illustrating the dramatic effect of proton on increasing the rate of nitrite reduction.

Such a maximal nitrite reductase activity at the hemoglobin P50 appears ideal for oxygen sensing and hypoxic vasodilation because this allosteric point is thermally, chemically, and electronically (referring to heme redox potential and equilibrium distribution of iron electrons) responsive to tissue metabolism. Additionally, a maximal reductase activity at P50 is biochemically consistent with a role in hypoxic vasodilation because physiological studies demonstrate an onset of hypoxic vasodilation at 40–60% hemoglobin oxygen saturation (79).

INTEGRATED BIOCHEMICAL PHYSIOLOGY

Nitrite appears to fit the requirements for a physiological mediator of hypoxic vasodilation because it maximally reacts with hemoglobin at 40–60% hemoglobin saturation, an oxygen tension (20–40 mmHg) significantly higher than that required for SNO-Hb deoxygenation, i.e., cysteine 93-ligated hemoglobins have very high oxygen affinities (6, 73). In the normal skeletal muscle circulation, oxygen tension decreases from the A1 caliber arterioles (100 μm diameter) to the A4 caliber arterioles (20 μm diameter) to values as low as 20 mmHg before the capillary circulation (91). These data suggest that much of the oxygen delivery occurs within the arterioles, allowing for anatomically linked oxygen delivery and vasomotor control. Additional mechanisms suggest that NO or ATP delivery to the capillary circulation produces retrograde intracellular propagation of vasodilating signal to the precapillary resistance vessels (84–86).

The question of how can nitrite be reduced by a single erythrocyte within a 10-s artery-to-arteriole-to-capillary transit time may be resolved by considering the red blood cell-oxygen tension equilibrium occurring within the microcirculation (Fig. 3). Intravital microscopy studies reveal that the arteries and arterioles do not contain a lonely isolated red blood cell drifting through the vasculature. Rather, the vessels are full of cells and

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FUTURE DIRECTIONS AND UNRESOLVED QUESTIONS

are essentially filled by a moving column of blood. Within this column of blood, the intracellular hemoglobin will rapidly deoxygenate from the artery to vein within ~10 s. At any anatomical site along the A1 to A5 arterioles, the hemoglobin concentration and oxygen saturation will be relatively constant, because as soon as one red blood cell moves downstream, a new one replaces it. The hemoglobin-oxygen saturation of this column of blood will shift up- or downstream depending on local tissue blood flow, oxygen content, and oxygen consumption.

From an equilibrium standpoint, there also exists a steady-state anatomical location within the circulation from artery to vein that has the greatest concentration of R₃ tetramers (R and T denote the respective oxy- and deoxytetrameric conformations, and the number denotes ligated oxygens), which possess the maximal nitrite reductase activity. At this anatomical location, there would always exist an equilibrium rate constant for nitrite reduction and an equilibrium concentration of nitrite and R₃ tetramer. We suggest, therefore, that as soon as one red blood cell moves downstream, a new one would replace it, thus preserving the concentration of nitrite and R₃ hemoglobin at that anatomical position. Thus there will be an increased nitrite reductase rate and increased NO concentration surrounding the blood vessel as the hemoglobin deoxygenates (Fig. 3). The anatomical position of this equilibrium NO concentration will be responsive to tissue metabolism and oxygen consumption by moving the R-to-T transition up- or downstream.

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