Evidence Mounts That Nitrite Contributes to Hypoxic Vasodilation in the Human Circulation

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Hypoxic vasodilation is a conserved physiological response to hypoxia that matches blood flow and oxygen delivery to tissue metabolic demand. This fundamental physiological process has been characterized for >100 years since the initial description by Roy and Brown in 1880. Hypoxic vasodilation requires a sensor mechanism that can detect a divergence in the normal relationship between delivered blood oxygen and tissue oxygen consumption. This hypothesis involves the generation of vasodilatory effectors that increase blood flow to maintain adequate tissue oxygenation. In short, hypoxic vasodilation requires hypoxia and/or pH sensing coupled to the release of a vasodilating signal. In mammalian species, the set point for hypoxic vasodilation occurs as the hemoglobin desaturates from 60% to 40%, around a partial pressure of oxygen ranging from 40 to 20 mm Hg. Despite the fact that the hypoxic vasodilation response was discovered almost 150 years ago, the identities of the oxygen sensor mechanism and the specific feedback vasodilator effectors remain uncertain. Although a number of mediators have been considered, including adenosine, nitric oxide (NO), ATP-sensitive potassium (KATP) channels, endothelium-derived hyperpolarizing factor (candidates include CO, H2O2, or ONOO−), and prostacyclin, the specific blockade of many of these pathways fails to completely inhibit hypoxic vasodilation.

The present study published by Maher and colleagues in this issue of Circulation provides compelling evidence in normal human volunteers that the circulating anion salt nitrite (NO2−) may be an effector of hypoxic vasodilation. They report that nitrite potently vasodilates the venous forearm circulation under resting conditions and potently vasodilates the arteriolar circulation during experimental hypoxia, achieved by systemic breathing of 11% oxygen. Their studies indicate that the vasodilatory effect of nitrite is maximal in the deoxygenated venous circulation and is greatly potentiated in the systemic circulation during hypoxia. These findings support a number of basic criteria necessary, although certainly not sufficient, for an effector of hypoxic vasodilation: The effector (1) must be naturally occurring, (2) must be metabolized or generated in response to tissue hypoxia, and (3) must potentiate vasodilation in response to hypoxia.

Large doses of nitrite given as an antidote for cyanide poisoning clearly produce hypotension in humans. Pharmacological concentrations of nitrite in the high micromolar range were shown to vasodilate rabbit aortic ring bioassays by Furchgott et al as far back as 1953 and were shown by Murad et al and Ignarro et al to activate guanylate cyclase and vasodilate in the mid-1970s and early 1980s. However, the high micromolar to millimolar concentrations necessary to achieve these effects in vitro contrasted with the low nanomolar concentrations present in mammalian blood; this disparity led to a dismissal of nitrite as a physiologically relevant vasodilator. Consistent with this assessment, studies published by Lauer and colleagues demonstrated that nitrite had no vasodilator activity when infused into the forearm circulation of normal volunteers, even at concentrations of 200 μmol/L. This observation appeared to close the door on the notion that nitrite was a physiological vasodilator.

Despite the apparent lack of bioactivity of nitrite in these studies, our group observed artery-to-vein gradients in nitrite across the human forearm, with increased consumption of nitrite during exercise stress. This suggested to us that nitrite was metabolized across the peripheral circulation, possibly in response to regional physiological deoxygenation of blood from artery to vein. Furthermore, when humans were exposed to inhaled NO gas, we observed an increase in peripheral forearm blood flow that was only associated with increases in plasma nitrite (a >90 nmol/L increase, with recent studies showing that inhaled NO gas increases plasma nitrite levels by as much as 1.5 μmol/L). To evaluate whether nitrite was vasodilatory in vivo, we infused nitrite into the forearm brachial artery of 28 healthy volunteers and, to our surprise, observed substantial vasodilation even without exercise stress. As now validated in the present study by Maher and colleagues, nitrite was potent in humans, increasing blood flow by 170% at 200 μmol/L in the forearm circulation and by 22% at 2.5 μmol/L. Even levels of 900 nmol/L produced vasodilation during exercise stress.

The mechanism of nitrite-dependent vasodilation appeared to be consistent with nitrite conversion to NO during physiological hypoxia in a process tightly coupled to hemoglobin deoxygenation. During nitrite infusions into the brachial artery of normal volunteers, we observed the artery-to-venous formation of iron-nitrosyl-hemoglobin (HbFe(NO)) suggesting that nitrite was being reduced to NO rapidly within one half circulatory time. This NO formation was inversely...
correlation with oxyhemoglobin saturation (ie, as hemoglobin deoxygenated, more NO was formed). We and others thus proposed that nitrite was reduced to NO along the physiological oxygen gradient and could contribute to hypoxic vasodilation.\(^1\)\(^2\)\(^4\) This hypothesis has been challenged by other investigators, who suggested that the observed increases in the venous oxygen tension during pharmacological nitrite infusions were not consistent with a hypoxic vasodilatory mechanism of activation.\(^14\) Therefore, the present studies by Maher et al\(^5\) are of particular relevance because they directly test the potency of nitrite in the human circulation during normoxia and hypoxia.

The physiological observations that nitrite infusions are tightly coupled to NO-hemoglobin formation are consistent with a known classic reaction between nitrite and deoxyhemoglobin to form NO, as described by Brooks\(^20\) in 1937 and by Doyle and colleagues\(^21\) in 1981:

\[
\text{NO}_2^- + \text{HbFe}^{+2} (\text{deoxygenated hemoglobin}) + \text{H}^+ \rightarrow \\
\text{NO} (\text{nitric oxide}) + \text{HbFe}^{+3} + \text{OH}^- 
\]

The NO formed in this reaction can then bind to another deoxyhemoglobin to form an NO heme bond (iron-nitrosyl-hemoglobin) or could escape the erythrocyte to mediate NO signaling.

\[
\text{NO} + \text{HbFe}^{+2} (\text{deoxygenated hemoglobin}) \rightarrow \\
\text{HbFe}^{+2-\text{NO}} (\text{iron-nitrosyl-hemoglobin})
\]

This chemical reaction possesses the “sensor” and effector properties necessary for hypoxic vasodilation: The reaction requires deoxyhemoglobin and a proton, providing oxygen and pH sensor chemistry, respectively, and generates NO, a potent vasodilator.

Biochemical and aortic ring studies indicate that nitrite/red blood cell–dependent vasodilation is initiated at an oxygen tension around the hemoglobin \(P_{\text{pH}}\) (\(P_{\text{O}_2}\) of 40 mm Hg for rat erythrocytes and 30 mm Hg for human erythrocytes), another requirement for a putative hypoxic vasodilator mechanism.\(^22\)\(^23\) 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 \(P_{\text{pH}}\).\(^22\)\(^23\)\(^24\) This maximal reductase activity of hemoglobin is allosterically regulated and peaks around the \(P_{\text{pH}}\) because of 2 unique and opposing properties of the hemoglobin tetramer. The first property involves oxygen binding to hemoglobin, which allosterically shifts hemoglobin to the R (relaxed, oxygenated) conformation. The heme groups of R state or oxyhemoglobin exhibit a decreased redox potential, making an electron transfer reaction from the heme to the bound nitrite more thermodynamically favorable.\(^24\) The second property that leads to a maximal reductase activity around the \(P_{\text{pH}}\) involves the role of the T-state or deoxygenated conformation of hemoglobin, which 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, which are plentiful in the T state, and oxyhemes with a higher intrinsic reactivity with nitrite, which are more plentiful in the R state, is met at 50% hemoglobin saturation (Figure).

The biochemistry of this reaction helps us to understand the vasodilatory phenomenon observed by Maher and colleagues.\(^5\) Using radionuclide plethysmography, they measured venous blood flow, and using strain gauge plethysmography, they measured arterial blood flow. Although these 2 measures are not strictly independent, they offer an estimate of the effect on the arterial versus the venous vascular beds. Interestingly, under normal physiological conditions the nitrite potently vasodilated the venous circulation. This could be consistent with the lower hemoglobin oxygen saturation in the venous circulation with more available deoxyhemes to bind and reduce nitrite. In the forearm arterial circulation at rest, deoxygenation of the hemoglobin in the resistance vessels is minimal. Indeed, in the antecubital vein of the resting human forearm, the hemoglobin oxygen saturation is 75% to 90%. It only drops to 50% with forearm exercise stress.\(^18\) This is in comparison to the coronary sinus hemoglobin oxygen saturation of 30% to 40% (\(P_{\text{O}_2}\) of 19 mm Hg). After Maher and colleagues exposed normal volunteers to 12% oxygen, which reduced arterial hemoglobin oxygen saturation to 83% to 88%, the potency of nitrite increased significantly in the arterial circulation but was unchanged in the venous circulation. In these experiments, hemoglobin oxygen saturation falls across the arterial-to-capillary circulation from 80% to 55%, exposing heme groups for nitrite reduction and apparently increasing the potency of nitrite-dependent vasodilation in the forearm arterial circulation.

The kinetic versus equilibrium considerations for nitrite reduction are interesting and are likely to have physiological and therapeutic implications. As illustrated in the Figure, in venous blood more deoxyhemes are available to reduce nitrite to NO (the brown line for deoxyhemoglobin concentration is shown to rise from artery to vein), but their reactivity is reduced because the hemoglobin will be largely in the T state (reactivity as measured by the bimolecular rate constant for nitrite reduction, which is shown by the green line in the Figure, drops from 6 mol/L·s\(^{-1}\) in the arterial circulation to 0.12 mol/L·s\(^{-1}\) in the venous circulation). As blood and nitrite pool in the venous circulation, the nitrite in venous blood will bind to T-state tetramers and slowly convert to NO. Although the kinetic rate of nitrite reduction is slower, owing to the low reactivity (bimolecular rate) of the T-state tetramer, the concentration of nitrite that can be reduced is greater, owing to the greater concentration of deoxyhemes available to bind and reduce nitrite. Therefore, equilibrium venous conditions produce higher concentrations of NO from nitrite reduction, albeit more slowly.

In the arterial circulation, things are quite different and would be hypothesized to more rapidly reduce a smaller amount of nitrite to NO (kinetic dominance). This is because arterial blood hemoglobin is fully oxygenated (R state) and with rapid deoxygenation will first generate R tetramer with loss of 1 or 2 oxygens (R\(^1\) and R\(^2\) tetramers; R and T denote the oxy and deoxygenated conformations, and the subscripted number denotes liganded oxygens) before conversion to the T state. These R\(^1\) and R\(^2\) tetramers have a high
reactivity with nitrite (high bimolecular rate constant), and although fewer hemes are available to bind nitrite, the rate of nitrite reduction will be maximal as they kinetically deoxygenate around the P50 (50% saturation with oxygen). This kinetic model is illustrated in the Figure by the rise in R3 tetramer during early deoxygenation (orange line) and the peak rate of nitrite reduction at the P50 (orange shaded region of the Figure). This biochemistry allows for rapid nitrite conversion to NO as red cells deoxygenate along the arterio-lar vascular tree. Indeed, in experimental systems, the faster one deoxygenates the red cell in the presence of nitrite, the faster vasodilation is observed.22

Maher and colleagues also propose that their results suggest a therapeutic opportunity for nitrite in the treatment of congestive heart failure. Indeed, venodilation would be expected to reduce cardiac preload, whereas arterial vasodilation reduces afterload and left ventricular wall stress. Unlike the organic nitrates (nitroglycerin), nitrite does not require enzymatic activation, is not subject to tolerance, and will preferentially vasodilate ischemic regions of the heart, thus limiting the risk of coronary “steal” syndrome.25

In conclusion, nitrite appears to be a naturally occurring circulating small molecule that is converted to NO and induces vasodilation with increasing potency during hypoxia. These findings are consistent with the proposal that nitrite may represent a physiological effector of hypoxic vasodilation and hypoxic signaling. Further physiological studies in animal models and humans with and without disease will be required to validate this thesis. Ongoing experiments exploring the role of other heme globins, such as myoglobin (which can be knocked out), as functional nitrite reductases should greatly advance our understanding of the role and importance of this signaling mechanism in physiology and therapeutics.26,27

Disclosures
Dr Gladwin is named as a coinventor on a National Institutes of Health government patent application for the use of sodium nitrite salts for the treatment of cardiovascular indications.

References


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