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COMMENTARY

Nitrite, NO and hypoxic vasodilation

Jason D Allen[1] and Andrew J Gow[2]

[1]*Department of Medicine, Duke University Medical Center, Durham, NC, USA, and* [2]*Department of Pharmacology & Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA*

The ability to deliver oxygen and other nutrients to working tissues at a rate acutely matched to demand is the quintessential function of the cardiovascular system. Thus, an understanding of the biochemical mechanisms involved in hypoxic vasodilation remains a major goal in vascular biology. Nitric oxide, its metabolites, and oxidation status are recognized as playing important roles in this process. Previous work examining how nitrite can be converted to bioactive nitric oxide (NO) under hypoxic conditions has focused on the role of the red blood cell and haemoglobin. In a recent issue of the *British Journal of Pharmacology*, Pinder *et al.* demonstrate that plasma nitrite, in the absence of haemoglobin, is capable of increasing the maximal dilation of rabbit aortic rings under hypoxic conditions. Furthermore, they demonstrate that this relaxation can occur with or without the endothelium. This observation, even if it is only a small proportion of the relaxant activity of nitrite, highlights how NO metabolites may be involved in a variety of mechanisms of vessel control.
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The paper by Pinder *et al.* is available from http://www3.interscience.wiley.com/journal/122526091/issue

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Abbreviations: eNOS, endothelial nitric oxide synthase; COX, cyclooxygenase

The ability to deliver oxygen and other nutrients to, along with the ability to remove waste products from, working tissues at a rate acutely matched to demand is the quintessential function of the cardiovascular system. It is logical therefore that several complex and often overlapping mechanisms are in place to protect this function. As early as 1880, it was reported that blood vessels may expand or contract in order to meet blood flow requirements (Roy and Brown, 1880). This model was further expanded upon in 1962 when it was shown that increased blood flow was tightly correlated with haemoglobin oxygen saturation in both spinal anesthetized and non-spinal anaesthetized dogs (Ross *et al.*, 1962). Although the authors suggested this effect was probably because vascular muscles would be unable to maintain a contraction as oxygen became scarce, rather than due to any vasodilator substance release, they nevertheless set the stage for the subsequent elucidation of hypoxic vasodilation. To date, a clear understanding of the biochemical mechanisms involved in these processes is a goal of vascular biology research.

Correspondence: Jason D Allen, Department of Medicine, Duke University Medical Center, DUMC 3022, Durham, NC 27710, USA. E-mail: j.d.allen@duke.edu
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Nitric oxide (NO), its metabolites and oxidation states are now recognized to play a significant role in the ability of red blood cells to control hypoxic vasodilation. The transport of NO equivalents from areas of high oxygen tension to those with low PO_2 is established, although the exact mechanisms and species involved are still vigorously debated.

More recently, hypoxic vasodilation has been shown in the absence of red blood cells suggesting that other, possibly integrated, overlapping or redundant pathways exist to ensure tissue perfusion. These may include waste products of metabolism such as adenosine, potassium, lactate and/or carbon dioxide among others. In this context, plasma nitrite may provide a bridge between red blood cell dependent and independent effects.

The vasodilatory properties of nitrite at supra-physiological levels have been recognized for decades (Furchgott and Bhadrakom, 1953); yet, the high micromolar concentrations required to elicit an effect limited its role in vascular physiology to that of an inactive product of NO oxidation. In fact, several groups (including ourselves) have used venous plasma nitrite as an index of short-term activity of the endothelial NO synthase (eNOS), because this product is relatively stable (in comparison with NO) and has a low background concentration (compared with nitrate).

The discovery of endocrine roles for NO equivalents in controlling hypoxic vasodilation (Jia *et al.*, 1996; Cannon

et al., 2001) has led to a search for particular NO metabolites that can control vascular function. One such metabolite is nitrite. Previously thought of as an 'NO-sink', nitrite has now been proposed to be transported in the blood and to be converted back to NO under hypoxic conditions (Lundberg *et al.*, 2008). During normoxia, localized vascular production of NO leads to increased plasma nitrite concentrations via NO oxidation, and during hypoxia, when the L-arginine/eNOS pathway may be dysfunctional, nitrite is reduced back to NO. This suggests a 'push-pull', complementary, system between the two pathways, enabling NO to be available to vessels across the oxygen gradient. This dual role of plasma nitrite has provocative implications for diet, exercise and cardiovascular homeostasis. It also raises the potential for the measurement of relative changes in plasma nitrite to represent a dynamic index of NO bioavailability and vascular health (Allen *et al.*, 2009).

Most previous studies examining the conversion of nitrite to bioactive NO have focused on the role of the red blood cell and haemoglobin (Cosby *et al.*, 2003). In a study published in a recent issue of the *British Journal of Pharmacology*, Pinder *et al.* (2009) have added a new facet to these concepts. They demonstrate that plasma nitrite (in the absence of haemoglobin) was capable of increasing the maximal dilation of rabbit aortic rings under hypoxic conditions. Furthermore, they demonstrate that this relaxation can occur with or without the endothelium. By selectively pharmacologically blocking potential pathways that may control this effect, they also show nitrite relies exclusively on NO as an intermediate in denuded vessels but can operate in both NO-dependent and, to a lesser extent an NO-independent manner in intact vessels.

The NO-dependent mechanism in both intact and denuded vessels appears to follow the classical endothelial-derived relaxing factor pathway as it is blocked both by the NO scavenger carboxy-PTIO and by the guanylate cyclase inhibitor, 1H-(1,2,4)oxadiazolo[4,3-a]quinoxalin-1-one. Several oxido-reductase enzymes have been proposed to be capable of using nitrite as an alternative electron acceptor to molecular oxygen and thereby forming NO. In this study, blockade of aldehyde oxidase decreased nitrite-induced vasodilation, but xanthine oxidase or NOS inhibition had no effect. Aldehyde oxidase is a novel potential nitrite reductase and the addition of another potential reductase activity demonstrates the degenerate nature of the system.

The NO-independent mechanism appears to rely on cyclooxygenase (COX) activity, as it was inhibited by indomethacin. However, it does not appear to involve prostacyclin synthase. COX activity, under the control of the endothelin $(ET)_B$ receptor, is a significant contributor to endothelial control of vascular tone. It is of note that this signaling pathway does not exist within the smooth muscle cell, which may explain the lack of effectiveness of indomethacin in the denuded vessel. This observation, even if it is only a small proportion of the relaxant activity of nitrite, highlights how NO metabolites may be involved in a variety of mechanisms of vessel control.

A note of caution must be added to the interpretation of these results as the experiments were performed at high doses of nitrite (~10 μM) using rings of isolated thoracic aorta and thus, while enhancing the pharmacological potential of this metabolite, they do not, *per se*, relate to the role of nitrite within normal vascular physiology in intact animals. What is clear is that these studies highlight the complexity of mechanisms involved in the control of vessel tension mediated by NO metabolites. Clearly, there is a need for further studies to understand both the biochemical mechanisms involved and the relevance of these mechanisms to vascular physiology and pathology.

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