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Invited Review

Mechanisms of nitrite bioactivation

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ABSTRACT

It is now accepted that the anion nitrite, once considered an inert oxidation product of nitric oxide (NO), contributes to hypoxic vasodilation, physiological blood pressure control, and redox signaling. As such, its application in therapeutics is being actively tested in pre-clinical models and in human phase I–II clinical trials. Major pathways for nitrite bioactivation involve its reduction to NO by members of the hemoglobin or molybdopterin family of proteins, or catalyzed dysproportionation. These conversions occur preferentially under hypoxic and acidic conditions. A number of enzymatic systems reduce nitrite to NO and their activity and importance are defined by oxygen tension, specific organ system and allosteric and redox effectors. In this work, we review different proposed mechanisms of nitrite bioactivation, focusing on analysis of kinetics and experimental evidence for the relevance of each mechanism under different conditions.

Contents

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Introduction

Even recently nitrite was considered inert and incapable of functioning as a vasodilator in the human circulation [1]. However, bioactivation of nitrite through formation of NO and other nitrogen oxides is now widely recognized and the importance of this pathway in the physiological control of blood flow and pressure accepted [2,3]. Despite agreement that nitrite is bioactive, the mechanisms responsible for bioactivation remain to be clearly defined and remain a source of controversy. In this review, we evaluate the studied mechanisms for reduction or bioactivation of nitrite (illustrated in Fig. 1) and discuss strengths and weaknesses related to their relevance in nitrite bioactivation.

There are now many pieces of evidence demonstrating bioactivity of nitrite. Nitrite administered to rats via an intragastric route lowered blood pressure [4]. Infusions of slightly supraphysiological levels of nitrite cause increased blood flow that are potentiated by hypoxia and low pH in humans [5,6]. Salivary nitrite has been shown to increase gastric mucosal blood flow and mucous thickness [7]. Physiologically relevant levels of nitrite have been shown to counter ischemic–reperfusion injury [8–10] that is likely mediated by effects on mitochondrial respiration [10]. In addition, nitrite has been shown to decrease platelet activation [11,12]. All of these effects are consistent with nitrite being reduced to NO and mediating NO-dependent signal transduction.

Numerous other examples of nitrite bioactivation have been demonstrated after initial conversion from nitrate by oral bacteria [13,14]. Physiological effects of increasing dietary nitrate include improving intestinal health [7], enhanced exercise performance [15–21], and acute reduction in blood pressure [22–26]. These effects are eliminated and nitrite levels decreased when volunteers either spit or use mouthwash [22,23], thereby implicating the importance of nitrate reduction to nitrite by oral bacteria. The fact that dietary nitrate is capable of raising plasma nitrite levels to a sufficient extent to elicit substantial physiological effects supports the notion that nitrite bioactivation plays a role in modulating normal biological function.

In this review we discuss the major pathways for nitrite reduction to NO and the evidence to support a role in physiology and disease. The major reductase enzyme pathways considered include the heme-based nitrite reductases, the molybdopterin enzyme nitrite reductases, and the nitrite anhydrase enzymes (Fig. 1).

History

In 1952 Furchgott and Bhadrakhom demonstrated the ability of nitrite to effect relaxation of pre-constricted aortic vessels, but the concentrations used where substantially higher than that found in physiological conditions (100 μM compared to normal levels of tens to hundreds of nanomolar) [27]. In humans, nitrite reduction to NO was discovered in expelled air and attributed to non-enzymatic reduction in the stomach [28]. This conversion of nitrite to NO in the stomach was suggested to play a role in controlling gut pathogens [29]. In 1995, nitrite was also shown to be reduced to nitric oxide in saliva [30]. In that same year, Zweier and colleagues demonstrated reduction of nitrite to NO in an ischemic heart model and attributed the reduction to non-enzymatic processes [31]. In 2000 Gladwin, Cannon and others observed an arterial-venous gradient in nitrite along with a substantial consumption occurring during exercise when nitric oxide synthase (NOS) is blocked in humans [32]. The authors suggested that nitrite may function as source of NO that is activated in hypoxia and acidic conditions to increase blood flow [32]. This suggestion would explain the observation by Classen and coworkers where intragastric administration of nitrite lowered blood pressure in a rodent model [4]. In 2001, Lundberg, Modine and colleagues showed that physiological levels of nitrite could effect vasorelaxation in rat aorta under acidic conditions of ischemia [33]. Then, in 2003, we showed that infusion of as little as a few micromolar nitrite increased blood flow in the human circulation in a NOS independent manner and this was potentiated when subjects performed exercise (Fig. 2) [5]. In a subsequent study, Gladwin and coworkers showed that infusion of physiological concentrations of nitrite caused increased forearm blood flow [6].

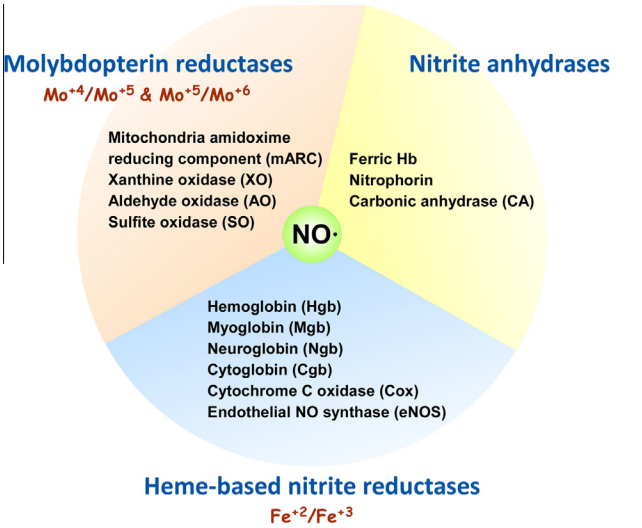

Fig. 1. Proposed protein or enzyme-based systems for nitrite reduction. The mechanisms shown here are based on those that are heme-based, molybdopterin-based, and those that function as nitrite anhydrases including nitrophorin [131,132].

Part 1. Nitrite reduction by heme proteins

Mechanism of hemoglobin mediated bioactivation

In our 2003 paper demonstrating nitrite's ability to increase blood flow we suggested that the mechanism may involve reduction to NO by deoxygenated Hb (deoxyHb) [5]. The chemistry for this reaction was first described by Brooks [34] and later elaborated upon by Doyle and colleagues [35] as well as others [36,37],

$$NO_2^- + HbFe^{2+} + H^+ \rightarrow NO + HbFe^{3+} + OH^- \quad (1)$$

$$NO + HbFe^{2+} \rightarrow HbFe^{2+} - NO, \quad (2)$$

where $HbFe^{2+}$ refers to deoxyHb (with the iron state in the reduced, +2, state), $HbFe^{3+}$ refers to methemoglobin (where the heme iron is oxidized), and $HbFe^{2+}$–NO refers to the NO adduct of hemoglobin, iron nitrosyl hemoglobin. The reduction of nitrite occurs as described in Eq. (1) and the dependence on low oxygen saturation and pH is manifest in the requirements for deoxygenated hemes and protons.

Interestingly, the rate of reduction has been found to depend on the allosteric state of the protein, with vacant hemes in an R-state Hb tetramer reacting about 100 times faster than those in the T-state [36–38]. The rate of NO production is thus maximal at partial oxygen saturations near the p50 of Hb (Fig. 3) [36,38]. The maximum rate of NO production at this oxygen pressure, taking a bimolecular rate constant of 1 M^{-1} s^{-1} [36], 0.01 M heme, and 100 nM nitrite would be 1 nM/s. This rate would increase linearly with a decrease in pH so that at a pH of 6.5 it would be about 8 nM/s. With higher plasma nitrate (such as that achievable after high oral