Graphical Review

Nitrite: A physiological store of nitric oxide and modulator of mitochondrial function

Sruti Shiva*

Department of Pharmacology & Chemical Biology, Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA 15261, USA

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ABSTRACT

Nitrite, long considered a biologically inert metabolite of nitric oxide (NO) oxidation, is now accepted as a physiological storage pool of NO that can be reduced to bioactive NO in hypoxic conditions to mediate a spectrum of physiological responses in blood and tissue. This graphical review will provide a broad overview of the role of nitrite in physiology, focusing on its formation and reduction to NO as well as its regulation of the mitochondrion—an emerging subcellular target for its biological actions in tissues.

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Introduction

While nitrite (NO$_2^-$) was for decades considered to be physiologically inert, it is now accepted that NO$_2^-$ represents a stable reservoir that can be reduced to bioactive NO in hypoxic conditions to mediate physiological signaling [1]. Concentrations of the anion are maintained at low micromolar levels in tissues (1–20 μM) and nanomolar levels in blood (100–200 nM) [2,3]. The majority of NO$_2^-$ is derived from the oxidation of NO Synthase (NOS)-generated NO. While this one electron auto-oxidation of NO proceeds relatively slowly (k = 2 x 10$^6$ M$^{-2}$ s$^{-1}$) compared to the two electron oxidation of NO to nitrate (NO$_3^-$) by heme proteins in the blood and tissue (k = 8 x 10$^7$ M$^{-1}$ s$^{-1}$), NO$_2^-$ formation can be catalyzed by the multicopper oxidase ceruloplasmin in the plasma or cytochrome c oxidase (CcOx) in tissues [4–6]. A smaller proportion (~30%) of NO$_2^-$ is derived from dietary sources. Nitrite itself is present in cured meats, however green leafy vegetable are a principal source of NO$_3^-$, which is reduced to NO$_2^-$ in the body by commensal bacteria in the oral cavity and the gastrointestinal tract and to a lesser extent by mammalian xanthine oxidoreductase (XOR) in the liver [7] (Fig. 1).

Once formed, NO$_2^-$ is reduced to bioactive NO through acidification or via reaction with a number of proteins possessing NO$_2^-$ reductase activity, including heme globins [8–10], molybdenum-containing enzymes [11,12], NOS [13], and components of the mitochondrial electron transport chain (ETC) [14–16]. While the reaction mechanism by which each of these systems reduce NO$_2^-$ has been elucidated to differing degrees, it is clear that NO$_2^-$ reduction by all mammalian reductases is optimized in conditions of hypoxia and acidosis (Fig. 2). Thus, NO$_2^-$ reduction represents a physiological mechanism by which NO production is sustained in hypoxic conditions, during which catalytic NO generation by NOS, which relies on oxygen as a substrate, is compromised (Fig. 1).
Perhaps the most well-characterized mammalian NO\textsubscript{2}/CO\textsubscript{2} reductases are the heme globins, which catalyze the following reaction:
\[
\text{deoxy(Fe}^{\text{II}}) + \text{NO}_2^- + \text{H}^+ \rightarrow (\text{Fe}^{\text{III}})^+ + *\text{NO} + \text{OH}^- 
\]

For hemoglobin (Hb), the rate of this reaction is regulated by the allosteric structural transition of the protein from its R (relaxed) to T (tense) state, such that the maximal rate of Hb-catalyzed NO\textsubscript{2}/CO\textsubscript{2} reduction occurs around the p50 of the protein (26 mmHg) [17]. This reaction has been implicated in the mechanism underlying hypoxic vasodilation. In tissues, the monomeric heme globins, myoglobin (Mb) and neuroglobin (Ngb), catalyze NO\textsubscript{2} reduction by the same reaction but at lower oxygen tensions (p50 Mb = 2.4 mmHg; p50 Ngb = 2.2 mmHg). Mb-dependent NO\textsubscript{2} reduction has been implicated in the protective effects of NO\textsubscript{2} after ischemia/reperfusion (I/R) in the heart as well as in vasodilation [18,19]. Neuroglobin, present in the brain and retina contains a hexa-coordinated group, which can be converted to a penta-coordinate heme capable of reducing NO\textsubscript{2} at a greater reaction rate than Mb and Hb. This transition of the heme coordination is regulated by the oxidation of two surface cysteine residues on the protein [10]. Molybdenum containing enzymes, of which XOR is best characterized, have been implicated in the mechanism underlying nitrite-dependent protection after I/R as well as protective vascular remodeling after vascular injury [12,20–22]. While the exact reaction scheme underlying XOR-mediated NO\textsubscript{2} reduction is unclear, it is known that this reaction occurs at the molybdenum cofactor of XOR and aldehyde oxidase [11,12]. Nitrite reduction by the mitochondrial ETC has been shown to occur in near anoxic conditions, predominantly at pH less than 7 and with relatively high (millimolar) concentrations of NO\textsubscript{2} [15]. Within the ETC, complexes III and IV predominate, while the hexacoordinate protein cytochrome c can reduce NO\textsubscript{2} to NO when it is converted to its pentacoordinate form, similarly to Ngb [14] (Fig. 2). Nitrite reduction by these enzymes with differing oxygen affinities, tissue distribution and rates of reduction, ensures NO generation and nitrosative modification of target proteins over a wide range of physiological hypoxia in the cell [1]. This leads to downstream signaling to induce a wide spectrum of biological responses including hypoxic vasodilation [8], stimulation of angiogenesis [23], modulation of glucose metabolism [24], augmentation of exercise efficiency [20], regulation of mitochondrial function [9,25,26] and tolerance to I/R [22,27–29] (Fig. 3).

It is now well-established that NO\textsubscript{2} mediates a number of beneficial tissue responses. While the downstream molecular signaling underlying these effects remains unclear, the...
The mitochondrion has emerged as a major sub-cellular target of NO\(_2\)/\(\text{CO}\). Accumulating evidence demonstrates that NO\(_2\)/\(\text{CO}\) differentially regulates mitochondrial function through the modulation of specific proteins within the organelle in both physiology and pathology (Fig. 4). The inhibition of mitochondrial complexes I and IV have been implicated in NO\(_2\)-mediated cytoprotection after I/R [18,26]. The mitochondrion plays a central role in the progression of I/R injury. During ischemia, ATP production is limited, contributing to the depletion of high energy phosphate stores. Upon reperfusion, overwhelming influx of oxygen into the...
respiratory chain results in excessive reactive oxygen species generation at complexes I and III and oxidation of critical proteins leading to opening of the mitochondrial permeability transition pore (PTP) as well as release of cytochrome c to initiate apoptosis [30,31]. Inhibitors of complex I have been demonstrated to attenuate I/R injury by limiting electron flow through the ETC at reperfusion, thereby limiting ROS generation [32]. It has now been demonstrated in a number of animal models of I/R that NO₂⁻ inhibits complex I activity specifically after ischemia [26,33,34]. This inhibition is attributed to the NO₂⁻-dependent S-nitrosation of complex I and results in an attenuation of mitochondrial ROS generation, as well as inhibition of PTP opening and cytochrome c release after I/R [26].

The reversible inhibition of cytochrome c oxidase (ccox; complex IV) has also been implicated in NO₂⁻-mediated protection after I/R [18]. Ccox, the terminal complex of the ecto to which oxygen binds at the copperheme₃₃ binuclear center and is reduced to water, is the primary target of NO within the mitochondrion. Binding of NO to the binuclear center excludes oxygen binding and inhibits respiration [35]. This NO-dependent inhibition of mitochondrial oxygen consumption is greater as oxygen tension is decreased and fully reversible [35]. We have demonstrated that Mb-dependent reduction of NO₂⁻ to NO results in the inhibition of ccox in the heart [9,18]. This inhibition of mitochondrial respiration potentially underlies the downregulation of metabolism, a protective phenomenon termed “short-term hibernation” that is responsible for conserving oxygen as well as high energy phosphates during prolonged ischemic episodes [18]. Once reperfusion commences, this inhibition is removed and metabolic function returns (Fig. 4A).

Nitrite dependent inhibition of ccox also potentially regulates responses to physiological hypoxia, such as that present in the muscle during exercise. Larsen and colleagues recently demonstrated that ingestion of NO₂⁻ decreased whole body oxygen consumption during exercise without changing maximal attainable work rate in human subjects [20]. This increase in exercise efficiency, which was associated with augmented plasma NO₂⁻ levels, has now been corroborated by a number of studies in various exercise models. While the underlying mechanism of this beneficial effect is not completely elucidated, a decrease in the rate of oxygen consumption due to proton leak and state 4 respiration in the skeletal muscle of subjects receiving NO₂⁻ was reported [25]. Further, the authors reported a NO₂⁻-induced decrease in the expression of uncoupling protein 3 (UCP-3) and the adenine nucleotide translocase (ANT), two proteins which facilitate proton leak [25]. Notably, numerous studies of respiratory control suggest that oxygen consumption by ccox can be inhibited to a certain degree without significantly affecting ATP production by the ETC [36,37]. Hence, it is possible that NO₂⁻-mediated inhibition of ccox could decrease oxygen consumption without negatively impacting ATP generation, contributing to the augmentation of the ratio of ATP generated per mole of oxygen consumed that was observed in subjects after NO₃⁻ ingestion.

In addition to modulating specific proteins within the mitochondrion, NO₂⁻ has also recently been shown to stimulate hypoxic mitochondrial biogenesis [38]. Treatment of cells with physiological levels of NO₂⁻ during chronic hypoxia induced a significant increase in mitochondrial number per cell. This effect is mediated through the classical mitochondrial biogenesis pathway involving the nitrite-dependent activation of AMP Kinase, Sirtuin-1, PPARγ-coactivator-1α and upregulation of mitochondrial transcription factors. This effect, observed both in vitro as well as in a rat model of restenosis, is associated with NO₂⁻-dependent protective vascular remodeling [38].

While the field of nitrite biology has advanced rapidly in the last decade, several challenges remain. The mechanisms underlying the regulation of individual nitrite reductases as well as the assessment of crossstalk between mammalian nitrite reductases are currently being elucidated. Ongoing study in a number of labs is identifying downstream targets through which nitrite mediates its effects. Future study will further delineate the role of nitrite reduction versus NOS-dependent NO generation in physiological NO signaling.

Acknowledgments

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