



Review

Mechanisms of nitrite reduction to nitric oxide in the heart and vessel wall

Jay L. Zweier*, Haitao Li, Alexandre Samouilov, Xiaoping Liu

Davis Heart and Lung Research Institute, The Division of Cardiovascular Medicine, Department of Internal Medicine, The Ohio State University College of Medicine, 473 West 12th Avenue, Columbus, OH 43210, USA

ARTICLE INFO

Article history:

Received 11 September 2009

Revised 10 December 2009

Available online 5 January 2010

Keywords:

Nitrite

Nitric oxide

Heart and vessels

Xanthine oxidase

Aldehyde oxidase

Electron paramagnetic resonance

Oxygen tension

ABSTRACT

Nitric oxide (NO) is an important regulator of a variety of biological functions, and also has a role in the pathogenesis of cellular injury. It had been generally accepted that NO is solely generated in biological tissues by specific nitric oxide synthases (NOS) which metabolize arginine to citrulline with the formation of NO. However, over the last 15 years, nitrite-mediated NO production has been shown to be an important mechanism of NO formation in the heart and cardiovascular system. Now numerous studies have demonstrated that nitrite can be an important source rather than simply a product of NO in mammalian cells and tissues and can be a potential vasodilator drug for cardiovascular diseases. There are a variety of mechanisms of nitrite reduction to NO and it is now appreciated that this process, while enhanced under hypoxic conditions, also occurs under normoxia. Several methods, including electron paramagnetic resonance, chemiluminescence NO analyzer, and NO electrode have been utilized to measure, quantitate, and image nitrite-mediated NO formation. Results reveal that nitrite-dependent NO generation plays critical physiological and pathological roles, and is controlled by oxygen tension, pH, reducing substrates and nitrite levels. In this manuscript, we review the mechanisms of nitrite-mediated NO formation and the effects of oxygen on this process with a focus on how this occurs in the heart and vessels.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Nitric oxide (NO) is a free radical endogenously produced in biological tissues and is an important regulator of numerous biological functions [1–4]. NO can also cause cellular injury via reaction with superoxide to form the potent oxidant peroxynitrite [5–7]. Although nitric oxide synthases (NOSs) have been generally considered to be the primary source of NO in biological systems, in 1995 we observed that prominent NO formation from nitrite occurs in heart tissues under conditions of intracellular acidosis [8]. A number of methods, including electron paramagnetic resonance, chemiluminescence NO analyzer, and NO electrode were utilized to measure, quantitate, and image nitrite-mediated NO formation. Now numerous studies have demonstrated that nitrite can be an important source rather than simply a product of NO in mammalian cells and tissues [8–24]. Results reveal that nitrite-dependent NO generation plays critical physiological and pathological roles, and is controlled by oxygen tension, pH, reducing substrates, and nitrite levels. Nitrite is currently undergoing or planned for clinical trials as a vasodilator drug in patients with cardiovascular diseases such as ischemic stress, sickle cell disease, coronary artery disease, and pulmonary hypertension. Therefore

fundamental understanding of the mechanisms of nitrite-dependent NO formation in the cardiovascular system is of great importance. In this manuscript, we review the mechanisms of nitrite-mediated NO formation and the effects of oxygen on this process with a focus on how this occurs in the heart and vessels.

Evidence for NO formation from nitrite in the heart

In the cardiovascular system, traditionally it was thought that NO is only produced in endothelial cells by endothelial nitric oxide synthase (eNOS) and then passively diffuses to either smooth muscle cells where it activates soluble guanylyl cyclase (sGC) or to the blood where it is mainly consumed by hemoglobin (Hb). However, in 1995, we first observed that nitrite can also be a prominent source of NO in heart tissues under conditions of ischemia with intracellular acidosis [8]. In the presence of isotopically labeled $^{15}\text{NO}_2^-$, NO production from ^{15}N -nitrite in anoxic heart tissue was measured by EPR spectroscopy with the spin trap iron *N*-methyl-D-glucamine dithiocarbamate (Fe^{2+} -MGD). ^{15}NO gives rise to a characteristic doublet ^{15}NO - Fe^{2+} -MGD spectrum, rather than the triplet observed with natural abundance ^{14}NO , enabling direct and selective detection of nitrite-derived NO formation. Hearts were labeled with 1 mM $^{15}\text{NO}_2^-$ for 1 min of normal perfusion or 1 min of perfusion prior to the onset of ischemia. In the normally perfused control hearts, $^{15}\text{NO}_2^-$ did not result in significant NO

* Corresponding author. Fax: +1 614 247 7845.

E-mail address: jay.zweier@osumc.edu (J.L. Zweier).

formation (Fig. 1A, left). In hearts which were labeled and then subjected to 30 min of ischemia; however, marked NO formation was observed with the appearance of a very large doublet signal (Fig. 1B, left). In matched experiments performed with addition of natural abundance $^{14}\text{NO}_2^-$, large NO triplet signals were seen (Fig. 1C, left). While the observed signal was similar to that in the absence of added nitrite, the intensity of this signal was much higher. These experiments demonstrated that nitrite is reduced to NO in the ischemic heart.

Experiments were also performed measuring nitrosyl-heme formation in ischemic heart tissue from hearts labeled with 1 mM $^{15}\text{NO}_2^-$. A prominent doublet nitrosyl-heme signal was seen due to the formation and binding of $^{15}\text{NO}_2^-$ to these proteins, further confirming that NO is generated from nitrite in heart tissue subjected to long periods of ischemia (Fig. 1B, right). With $^{14}\text{NO}_2^-$ a similar magnitude triplet NO signal was observed (Fig. 1A, right).

The rate of reduction of nitrite to NO is pH dependent. During myocardial ischemia, marked intracellular acidosis occurs, and pH values can fall to levels of 6.0 or below [21]. The low pH along with a highly reduced state under hypoxia could cause the rapid reduction of nitrite to NO. This process was shown to occur following myocardial ischemia as in the clinical setting of heart attack and it was extrapolated that nitrite disproportionation and reduction would be a generalized phenomena in any tissue under conditions of ischemia or shock, where poor perfusion and accompanying acidosis occur [8,21].

Methods of measuring, quantitating and imaging nitrite-mediated NO formation

NO is paramagnetic and binds with high affinity to the water-soluble spin trap, Fe^{2+} -MGD, forming a mononitrosyl iron complex with characteristic triplet spectrum at $g = 2.04$ with hyperfine splitting $a_N = 12.8$. From the intensity of the observed spectrum, quantitative measurement of NO generation can be performed [8,21]. As noted above, this technique has been applied to measure nitrite-mediated NO generation and with ^{15}N -labeled nitrite it can be used to isotope trace the process of nitrite-mediated NO formation [16–18,25–30].

The advantage of electrochemical NO sensors is their ability to directly detect NO concentration in solution or in biological samples with high sensitivity and a detection limit of a few nanomolar [31,32]. This advantage allows NO electrodes to be an excellent tool for studying NO reaction kinetics, especially in biological

samples. NO electrode studies were used to investigate enzyme catalyzed nitrite reduction as from XO and this method avoids any possible perturbation as might be caused by the presence of a trap [16–18]. The electrochemical detector continuously records the current through the working electrode, which is proportional to the NO concentration in the solution.

The real-time rate of the NO production can be measured using a chemiluminescence NO analyzer. In the analyzer, NO is reacted with ozone forming excited-state NO_2 , which emits light [33]. Mixing of reagents and separation of NO from the reaction mixture can be done at controlled temperature in a glass-purging vessel equipped with heating jacket. The release of NO is quantified by analysis of the digitally recorded signal from the photo-multiplier tube. Since the NO analyzer registers the flux of NO in the purging gas mixture it measures the rate of NO production from the system [33]. The rates of NO formation derived from Xanthine oxidoreductase (XOR), aldehyde oxidase (AO), cytochrome P450, rat tissues, and blood have been measured using the NO analyzer [16–18,26,34–36].

With the development of EPR imaging (EPRI) methods and low frequency EPR instrumentation, it has become possible to spatially map the presence, quantity, and location of free radicals and paramagnetic species in both *in vivo* and *ex vivo* biological tissues [37,38]. With the use of ^{15}N -nitrite, spatial mapping of nitrite-derived NO was performed in the ischemic heart [39]. A series of rat hearts were loaded with Fe^{2+} -MGD and ^{14}N - or ^{15}N -nitrite and subjected to global no-flow ischemia. After 60 min, three-dimensional projection data were acquired and image reconstruction performed. Fig. 2A shows 3D images obtained in these hearts. Both the ^{14}N - and ^{15}N -labeled hearts exhibited similar images of NO distribution. The ^{15}NO image, however, was somewhat sharper due to enhanced sensitivity and better resolution of the hyperfine doublet structure. The images clearly show that NO is formed throughout the left ventricular (LV) myocardium. The NO image enabled visualization of the external shape of the epicardium, right ventricular (RV) myocardium and internal endocardial surface of the LV and LV chamber. The concentrations of NO in the RV, however, were much lower with levels only 20–25% of those seen in the LV wall. To understand the spatially resolved time course of NO generation in the ischemic heart, EPRI of NO formation was performed as a function of ischemic duration. A series of images were performed every 5 min during ischemia for a total period of 60 min. Representative slices of longitudinal and transverse cuts through the LV are shown in Fig. 2B. It was observed that the NO

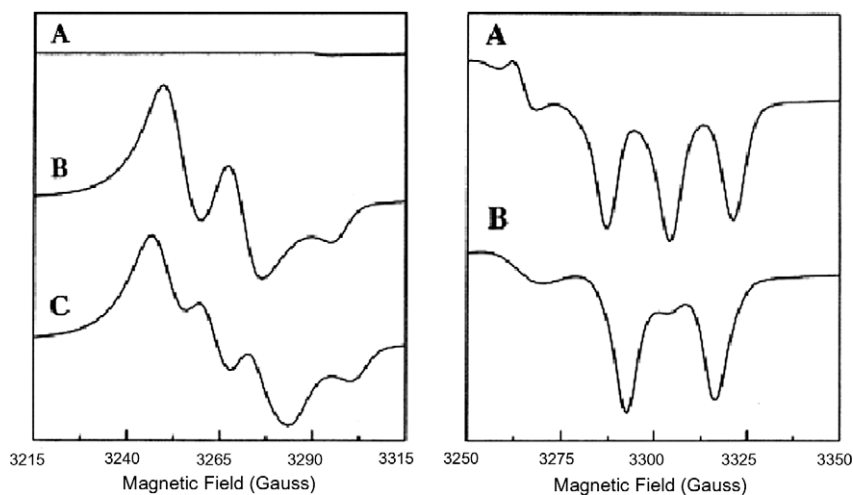


Fig. 1. EPR spectra of NO formation in the heart from nitrite. Hearts were prelabeled with 1 mM ^{15}N - or ^{14}N -nitrite. The left panel shows spectra from hearts with Fe -MGD; (A) before ischemia; (B) 30 min of ischemia with ^{15}N -nitrite; (C) 30 min ischemia with ^{14}N -nitrite. The right panel shows nitrosyl-heme formation; (A) with ^{14}N -nitrite; (B) with ^{15}N -nitrite.

Download English Version:

<https://daneshyari.com/en/article/2001235>

Download Persian Version:

<https://daneshyari.com/article/2001235>

[Daneshyari.com](https://daneshyari.com)