Nitric Oxide 35 (2013) 175–185

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Review

Nitric oxide, a protective molecule in the cardiovascular system

Jing Lei *, Yoram Vodovotz, Edith Tzeng, Timothy R. Billiar

Department of Surgery, University of Pittsburgh, United States

ARTICLE INFO

Article history:
Received 5 May 2013
Revised 2 September 2013
Available online 1 October 2013

Keywords:
Nitric oxide
Endothelial cell
Smooth muscle cell
Blood vessel injury
Intimal hyperplasia
Transplant vasculopathy

ABSTRACT

Nitric oxide (NO) is an intra- and inter-signaling molecule that regulates vessel dilatation, neuronal transmission, cardiac contraction, immunomodulation, and stem cell differentiation and proliferation. NO plays an important protective role in the cardiovascular system. NO inhibits smooth muscle cells proliferation and migration; enhances proliferation and migration of endothelial cell and inhibits apoptosis; suppresses platelet aggregation; and prevents platelet, leukocyte and monocyte adhesion to endothelium. NO exerts an inhibitory effect on the development of intimal hyperplasia in mechanically or immunologically injured vessel. New therapeutic approaches aimed at enhancing NO bioavailability or assisting delivery of NO locally may help patients with cardiovascular disease.

Contents

Introduction

Nitric oxide (NO) is an intra- and intercellular signaling molecule that plays important roles in many physiological and pathological processes, including vasodilatation, neuronal transmission, immunomodulation, cardiac contraction, inhibition of platelet aggregation, stem cell differentiation and proliferation [1–6,151,152]. Its protective role in the cardiovascular system was traced back to 1867 when Brunton used amyl nitrite to treat angina pectoris [157]. In 1937, Weiss et al. investigated the cardiovascular effect of nitrite in human subjects [158]. But for over one hundred years, it was not known that it was NO that played the role and NO was regarded as toxic gas. Until the early 1980's, the critical role of NO in the cardiovascular system was identified, and the Nobel Prize in Physiology or Medicine was awarded to Drs. Robert F. Furchgott, Louis J. Ignarro and Ferid Murad for their seminal discoveries of NO as a signaling molecule in blood vessels. Since the identification of NO as the endothelium derived relaxing factor, numerous other protective properties of NO in the cardiovascular system have been characterized. This review will explore the protective roles of NO in

* Corresponding author. Address: F1281.3 Presbyterian Hospital, 200 Lothrop Street, Pittsburgh, PA 15213, United States. Fax: +1 412 647 3247.
E-mail address: jinglei2009@gmail.com (J. Lei).

1089-8603/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.niox.2013.09.004

blood vessels, focusing on a description of the early discoveries in the field.

NO properties

NO is produced endogenously through the action of NO synthases (NOSs) from the substrate L-arginine. It has a half life measuring on the order of seconds in biological systems and it is highly diffusible, allowing it to quickly target adjacent cells. There are three mammalian NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). The expression patterns and enzyme activity of the three isoforms are summarized in Table 1. The varied expression and activity patterns of the NOS enzymes uniquely suit the different functions of the NO generated from each isoform in normal physiologic functions and in disease states. NO can also be delivered pharmacologically by NO donor compounds, e.g. S-nitroso-N-acetylpenicillamine (SNAP), that have different release profiles and tissue targeting abilities. More recently, it has also been determined that nitrite, previously recognized as an inert, stable end-product of NO metabolism, can actually serve as an important substrate for NO production through nonenzymatic reduction, or deoxyhemoglobin [146], or enzymatic reaction of oxidoreductases such as xanthine oxidoreductase [159–161]. Under physiological condition, nitrite does not function as an NO storage pool. Nitrite concentration is 150–1000 nM in plasma and >10 μm in aortic tissue [146]. To achieve a vasodilation effect, a large dose of exogenous nitrite (36 μmol/ml, total 5 ml) was required to be delivered into human subjects and nitrite concentration in local vein reached to about 200 μM [146]. However, under hypoxic condition, endogenous nitrite reacts with deoxyhemoglobin and releases NO for vessel dilation. This reaction is as following:

$NO_2^- + HbFe^{2+}$ (deoxyhemoglobin) $+ H^+ \rightarrow HbFe^{3+}$ (methemoglobin) $+ NO + OH^-$ [146]. A high concentration of methemoglobin in plasma will reverse the reaction direction and decrease the generation of NO. In addition, nitrite can also be reduced to NO by the enzyme xanthine oxidoreductase. Administration of nitrite in rats resulted in reduced pulmonary and arterial systemic pressure and enhanced cardiac output. In contrast, these effects were attenuated by the xanthine oxidoreductase inhibitor allopurinol [162,163].

NO largely functions through its interactions with heme moieties in a variety of enzymes. One of its most noted targets is soluble guanylyl cyclase (sGC). Binding of NO to the heme in sGC activates the enzyme to produce cGMP which is the second messenger responsible for the vasodilation associated with NO. NO can also bind to cysteines and thiols through nitrosation reactions. These modifications are important post-translational changes that alter and regulate protein function. In pathophysiologic states, NO reacts with reactive oxygen species to form peroxynitrite ($ONOO^{\bullet -}$) and other reactive nitrogen molecules which can lead to nitration reactions that then mediate cell injury and death. Nitrotyrosine is one such product of nitration from peroxynitrite. It is associated with numerous pathologic conditions and has been found in diseased tissues. Peroxynitrite is in general regarded as a cytotoxic molecule, while in certain circumstances it can also provide vasodilatory effect, inhibit leukocyte-endothelial cell adhesion, or reduce myocardial infarct size [164–167].

Vasculoprotective roles of NO

NO has been shown to exert many vasculoprotective roles in the cardiovascular system. These protective effects can be traced back to the cellular effects of NO on the individual cell types that are most important in cardiovascular disease, namely vascular smooth muscle cells (SMC), endothelial cells (EC), platelets and inflammatory cells. These protective roles will be described in detail in the following sections.

NO inhibits SMC proliferation and migration

The inhibitory effect of NO on SMC proliferation was first reported in 1989 by Garg et al. [13]. Three NO donors, SNAP, sodium nitroprusside (SNP), and isosorbide dinitrate (ISDN), inhibited SMC proliferation in a dose-dependent pattern. The ability of these donors to block proliferation was reversed when hemoglobin, a strong NO scavenger, was added and demonstrated that the effect of these donors was mediated through NO release. This anti-proliferative effect could be mimicked with the cGMP analog, 8-bromo-cGMP, indicating that NO mediated suppression of cell proliferation was mediated through cGMP. In contrast, SNAP-induced suppression of cell proliferation was accelerated when superoxide dismutase (SOD) was added. SOD catalyzes the dismutation of superoxide (oxidation of one superoxide and reduction of another one to form oxygen and hydrogen peroxide), preventing the interaction of NO and superoxide. Without SOD, NO rapidly reacts with superoxide at a diffusion limited rate to form peroxynitrite.

Cornwell et al. [14] further showed that the cytokine IL-1β or NO donors, SNAP or SNP, inhibited PDGF-induced SMC proliferation. NO increased cGMP levels which activated cAMP kinase. However, NO did not change cAMP levels. The cAMP kinase inhibitor (R)p-bromoadenosine 3′,5′-cyclic monophosphorothioate (Rp-8-BrcAMP[S]) reversed NO induced inhibition of SMC proliferation. However, the cyclic GMP kinase inhibitor [(R) p-bromoguanosine 3′,5′-cyclic monophosphorothioate (Rp-8-BrcGMP[S]) did not exhibit this inhibitory effect. Garg and Cornwell's results demonstrate that NO inhibits SMC proliferation through cGMP mediated cAMP

Table 1
Three types of NOS isoforms.

NOS isoforms	Expression characteristics	Cell typed expression	Enzyme activity
eNOS	Constitutive	Mainly in endothelial cells, also in smooth muscle cells, cardiomyocytes, bone cells and neuron	Ca^{2+}-dependent, generates NO in nanomolar level [5,11,12]. Phosphorylation at Ser1177, Ser615, Ser633, Tyr81 (bovine Ser1179, Ser617, Ser635, Tyr83) enhances activity [187–190]; phosphorylation at Ser114, Thr495 and Tyr657 inhibits activity [191–194]
nNOS	Constitutive	Mainly in neurons, also in pancreas and kidney	Ca^{2+}-dependent, generates NO in nanomolar level. Phosphorylation at Ser1417 (rat Ser1412) [195], Ser1451 [196] enhances activity; phosphorylation at Ser852, Ser746, Thr1301 (rat Ser847, Ser741, Thr1296) attenuates activity [197–199]
iNOS	Not expressed under basal conditions	Inducible in many cell types by stimuli, including inflammatory cytokines, microbes, microbial products (LPS) and mechanical perturbation [7–10]	Not dependent on Ca^{2+}, iNOS generates NO in micromolar concentration range for long periods of time [2,3,5]. Phosphorylation at Ser745 [200] or Tyr1055 [201] enhances iNOS activity or stabilizes iNOS half life; Phosphorylation at Tyr151 [202] attenuates activity