Nitric Oxide 49 (2015) 8–15

Contents lists available at ScienceDirect

Nitric Oxide

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

Review

Compartmentalized nitric oxide signaling in the resistance vasculature

Stephanie M. Mutchler [a, b], Adam C. Straub [a, b, *]

[a] Heart, Lung, Blood and Vascular Medicine Institute, USA
[b] Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15216, USA

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
15 May 2015
Accepted 20 May 2015
Available online 28 May 2015

Keywords:
Nitric oxide
Resistance artery
Vascular tone
Myoendothelial junction
Hemoglobin

ABSTRACT

Nitric oxide (NO) was first described as a bioactive molecule through its ability to stimulate soluble guanylate cyclase, but the revelation that NO was the endothelium derived relaxation factor drove the field to its modern state. The wealth of research conducted over the past 30 years has provided us with a picture of how diverse NO signaling can be within the vascular wall, going beyond simple vasodilation to include such roles as signaling through protein S-nitrosation. This expanded view of NO's actions requires highly regulated and compartmentalized production. Importantly, resistance arteries house multiple proteins involved in the production and transduction of NO allowing for efficient movement of the molecule to regulate vascular tone and reactivity. In this review, we focus on the many mechanisms regulating NO production and signaling action in the vascular wall, with a focus on the control of endothelial nitric oxide synthase (eNOS), the enzyme responsible for synthesizing most of the NO within these confines. We also explore how cross talk between the endothelium and smooth muscle in the microcirculation can modulate NO signaling, illustrating that this one small molecule has the capability to produce a plethora of responses.

1. Introduction

Blood pressure and tissue perfusion are regulated in part by the arterioles just upstream of capillaries known as resistance arteries. This vast network of vessels reacts to a constant flurry of endocrine, paracrine and autocrine signals to maintain homeostasis in response to the ever-changing needs of the local environment while regulating the pressure of the system as a whole. An architectural detail facilitating constant cell–cell communication in resistance arteries is the myoendothelial junction (MEJ), a point of connection between endothelial and smooth muscle cells. Originally, it was believed that the thickness of the internal elastic lamina would preclude any direct contact between the two cell types, but in 1957, Moore and Ruska utilized transmission electron micrographs to describe MEJs at the ultrastructural level as fenestrations of the internal elastic lamina through which endothelial cell protrusions could extend [1]. Over the next 50 years, research into the functional roles of these projections revealed their importance in second messenger communication and electrical signaling within the vascular wall. MEJs tend to have a greater presence in arteries with a smaller luminal size [2,3], which allows these vessels a faster endothelial response to second messengers originating in the smooth muscle [4] by providing a platform for the organization and localization of signaling proteins [5].

One of the most important signaling molecules within the vasculature is nitric oxide (NO). NO is a short-lived molecule with diverse biological functions. Thought to evolutionarily precede both the presence of oxygen in the atmosphere and the development of heme-containing proteins, NO is a ubiquitous signaling molecule [6]. It plays an important role in neurotransmission [7,8], regulation of vascular tone and vasodilation [9,10], and regulation of both gene transcription [11,12] and mRNA translation [13,14]. Because of NO's role in maintaining proper signaling, its dysregulation can contribute to pathologies such as stroke, multiple sclerosis, Alzheimer's disease, and atherosclerosis [15,16].

2. Regulation of NO production: the dynamic modulation of eNOS

The nitric oxide synthases are the heme-containing enzymes responsible for NO production. Three isoforms, neuronal (nNOS), inducible (iNOS) and endothelial (eNOS), share the common

* Corresponding author. University of Pittsburgh School of Medicine, Vascular Medicine Institute, E1254 Biomedical Science Tower, 200 Lothrop St., Pittsburgh, PA 15216, USA.
E-mail address: astraub@pitt.edu (A.C. Straub).

http://dx.doi.org/10.1016/j.niox.2015.05.003
1089-8603/

characteristic of utilizing L-arginine, molecular oxygen, and NADPH as substrates to produce NO and L-citrulline [17]. The heme site is ultimately responsible for this conversion, which occurs in two steps: first, NOS hydrolyzes L-arginine to $N^{(\omega)}$-hydroxy-L-arginine, and second, the NOS enzyme oxidizes $N^{(\omega)}$-hydroxy-L-arginine to L-citrulline and NO [18,19]. eNOS is of particular importance in the vasculature, and its signaling capacity is in part due to its ability to interact with multiple protein partners, both in an inhibitory and permissive manner (for detailed review see Ref. [20]). Post-translational modifications allow for eNOS modulation through the actions of several signaling cascades, and the comprehensive regulation of this one protein allows for differential signaling through the same chemical messenger.

2.1. Post-translational modifications on eNOS

In order for eNOS to effectively signal, it must be located in a position easily accessible to G-protein coupled receptors, growth factor receptors and calcium regulatory proteins. The caveolae provide such an environment [21], and eNOS can be targeted to this specific signaling domain by post-translational modifications. First, myristoylation of the N-terminal glycine occurs co-translationally [21], targeting the protein to the membrane and priming it for palmitoylation at cysteines 15 and 26 [22]. This reversible modification allows for firm anchorage within the lipid bilayer, but depalmitoylation can occur with prolonged stimulation [23], allowing eNOS to travel back into the cytosol, regulating its activity via its cellular localization.

Once eNOS is at the membrane, a number of phosphorylation events can occur to either promote activity or inhibit it (for detailed review see Ref. [24]). Phosphorylation at Ser1177 increases the sensitivity of eNOS to calcium by allowing calmodulin, a protein which binds directly to the enzyme, to remain associated and also by enhancing the rate of electron transfer within the reductase domain of eNOS [25,26]. When the serine at this position is replaced with aspartic acid to mimic phosphorylation, the enzyme becomes constitutively active [27]. Ser617 and Ser635 also increase the activity of eNOS either directly or by sensitizing the enzyme to calcium [28]. Inhibition of the enzyme occurs with phosphorylation events at Thr495 and Ser116. Thr495 phosphorylation limits the binding of calmodulin and shifts eNOS towards the production of superoxide rather than NO [29,30].

Because phosphorylation at these different sites can be induced by a variety of kinases including protein kinase B (PKB), cyclic AMP-dependent protein kinase (PKA), AMP-activated protein kinase (AMPK), and calcium/calmodulin-dependent protein kinase II (CaM kinase II) [31,32], the phosphorylation state of the enzyme is dynamic and may help to control the level of activity rather than just serving as an on/off switch. For example, VEGF acts to stimulate eNOS activity by causing the dephosphorylation of Ser116, but this event is not seen with many other eNOS agonists [33]. This suggests that phosphorylation may fine tune the production of NO and modulate the ability of the enzyme to associate with other proteins and cellular elements such as the cytoskeleton. Because this phosphorylation profile may have a greater consequence than simply turning the enzyme "on or off", deeper investigation into the exact phosphorylation events that occur in disease is important, as it may provide essential clues as to the exact level of dysregulation taking place.

In this same vein, Rafikov et al. argue that the simple on/off model does not give a complete picture of eNOS regulation. They present evidence for the existence of a "flexible arm" which operates in response to the redox state of eNOS, allowing the enzyme to cycle into an active versus inactive state in accordance with the redox potential of the surrounding environment [34]. eNOS does contain an autoinhibitory element (AIE) which, together with the C-terminal tail, modulates the phosphorylation status at Thr495 and Ser1177. When this AIE was deleted, eNOS activity increased under basal conditions possibly due to eNOS's greater ability to interact with other activating proteins [35].

It is known that multiple extracellular signals, such as acetylcholine [36] and vascular endothelial growth factor [37], can stimulate eNOS activity. Conversely, other signals can inhibit eNOS activity such as thrombospondin-1 (TSP1). TSP is a large glycoprotein first identified as a major product of platelets [38]. A typical matricellular protein, TSP1 is secreted into the matrix in times of need, but is broken down or taken back up by cells in order to regulate its function. When expressed, TSP1 interacts with several receptors on endothelial cells [39–41], and regulates eNOS function and NO signaling. TSP1 is known to regulate NO signaling through two of its receptors, CD36 and CD47, however CD47 appears to be more imperative for signaling at physiological levels of NO [42,43]. CD47 has been shown to associate with caveolin-1, a negative regulator of eNOS, in lung endothelial cells; upon activation of CD47 by TSP1, this association is disrupted leading to a decrease in caveolin-1 and increase in eNOS superoxide production [44]. Furthermore, TSP1 inhibits eNOS phosphorylation at Ser1177 in endothelial cells, preventing acetylcholine-induced vasorelaxation [45] Fig. 1.

2.2. eNOS binding partners

2.2.1. Membrane associated proteins: Caveolin-1 and endoglin

Caveolae are invaginations in the membrane where signaling molecules can congregate in order to improve efficiency of signaling. These membrane regions are rich in both caveolin-1 and caveolin-2 in the endothelium, with their incorporation into the membrane being dependent on cholesterol [46,47]. eNOS is targeted to caveolae by myristoylation and palmitoylation, but once it is localized to these specialized areas of the membrane, it is negatively regulated by its direct association with caveolin-1 [48]. Caveolin-1 acts to inhibit eNOS activation by sterically blocking the binding site for calmodulin [23], but it also regulates signaling by inhibiting signaling proteins that activate eNOS.

Endoglin, also known as CD105, is another membrane protein enriched within caveolae. Unlike caveolin, endoglin supports eNOS activity by promoting its association with hsp90 and inhibiting its membrane disassociation and degradation [49]. Endoglin is part of the transforming growth factor beta (TGF-β) receptor complex; TGF-β1 leads to increased vasodilation by endoglin-dependent transcriptional increase in eNOS levels mediated by ALK5 and its downstream substrate Smad2 [50]. Recent work demonstrated that loss of endoglin in endothelial cells causes eNOS uncoupling and up-regulation of TSP-1 [51]. Because TSP-1 converts latent into active TGF-β1 as well as suppresses NO signaling, endoglin expression is critical for endothelial cell function and loss of expression may be a major contributing factor to vascular dysfunction.

2.2.2. Calmodulin

Calcium is required for eNOS activation [52], with an increase in intracellular calcium levels being the spoint of convergence for several eNOS activation pathways. Calcium exerts its influence over activity via the calcium modulated protein calmodulin which reversibly binds to eNOS to promote dimerization of the protein [53] while also facilitating transfer of electrons between the reductase and oxygenase domains [54]. Calmodulin binding allows for eNOS to be freed from its inhibitory interaction with caveolin-1, facilitating the recruitment of other factors which increase activity [20].