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Compartmentalized nitric oxide signaling in the resistance vasculature

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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.

Keywords

nitric oxide; resistance artery; vascular tone; myoendothelial junction; hemoglobin

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

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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 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^ω-hydroxy-L-arginine, and second, the NOS enzyme oxidizes N^ω-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 [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 de-palmitoylation 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 [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].

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- β 1 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- β 1 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 point 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].

2.2.3 Heat Shock Protein 90—Heat shock protein 90 (hsp90) is a known chaperone, assisting in protein folding and maturation events. In the case of eNOS, it appears to play a significant role, aiding in dimerization, activation, and stabilization. Upon tyrosine phosphorylation induced by eNOS agonists, hsp90 becomes associated with eNOS [55–57]. This interaction increases calmodulin binding, thus sensitizing the system to rises in calcium levels [58]. Recent work has shown that hsp90 is required for dimerization, and it is this dimerization that is the predominant condition for eNOS phosphorylation and activation rather than the modifications being the permissive event for dimerization [59]. Hsp90 promotes these phosphorylation events by potentially increasing the binding affinity for eNOS and Akt and causing a conformational shift that unmask sites for modification [60,

61]. Several eNOS agonists stimulate rapid eNOS-hsp90 interaction and blockade of this partnership limits NO production and vasorelaxation [55, 62] with pathological conditions leading to a decrease in the association of these two proteins [63, 64].

2.2.4 Cytoskeletal Interactions: NOSIP and NOSTRIN—eNOS interacts with actin, potentially through a direct association with its oxygenase domain [65], but also by its interaction with other proteins capable of interacting with cytoskeletal elements. Binding of G-actin and β -actin by eNOS has been shown to increase NO production either by increasing activity of the enzyme or switching the output from superoxide to NO ([66]). Interaction with the cytoskeleton not only modulates the activity of eNOS, it also allows for trafficking of the protein from the plasma membrane to other intracellular structures.

Two proteins involved in this trafficking process are *eNOS Trafficking Inducer* protein (NOSTRIN) and *eNOS Interacting Protein* (NOSIP) [67, 68]. NOSTRIN, a 58kDa protein with high expression in the endothelium [69, 70], has a C terminal coiled-coil motif that may trimerize and serve as a platform for binding of several proteins [71]. Because NOSTRIN also associates with caveolin-1, it is believed that NOSTRIN-dependent movement of eNOS proceeds through a specialized endocytosis of caveolar endosomes [70]. This internalization depends greatly on the actin cytoskeleton, and NOSTRIN may provide the necessary link for two proteins crucial to the process: dynamin-2 and N-WASP [71].

NOSIP is a 34 kDa protein that binds to the oxygenase domain of eNOS to assist in translocation from the plasma membrane to intracellular membranes. NOSIP competes with caveolin-1 for binding to eNOS due to their shared binding domain. Overexpression of NOSIP leads to decreased NO production, possibly because of this competition, as removing eNOS from the caveolae limits its interaction with co-localized effectors [67].

These interactions with the cytoskeleton may provide a reason as to why mechanical forces such as shear stress induce eNOS activation [72]. Shear stress has been shown to increase Ser1177 phosphorylation [25] and increase NO production as measured through S-nitrosation of at least 12 major proteins in endothelial cells [73].

3. The Myoendothelial Junction and NO Signaling

Because NO has a short half-life and rapid diffusion rate, the effectiveness of eNOS is in part based on its ability to localize to its target. The MEJ forms a bridge from the endothelial cell to the smooth muscle cell, where various proteins and cellular structures localize including caveolae [74] and cytoskeletal components [75, 76] providing an ideal environment for eNOS signaling. Indeed, eNOS itself is enriched within the MEJ in a number of vascular beds, allowing it to effectively target its receptor, sGC, within the smooth muscle cell [74]. These signaling microdomains provide their own calcium store via the endoplasmic reticulum which extends into the cellular bridges [75, 77], allowing for release after inositol triphosphate (IP₃) receptor or transient receptor potential 4 (TRPV4) channel activation [76, 78]. This localized calcium provides the necessary trigger for eNOS activation, and allows for highly regulated production of NO within the cell. However, the MEJ does not simply provide a pathway for carrying NO from one cell type to the other

within the vascular wall; it is a carefully regulated environment, with systems at the MEJ allowing for modulation of second messenger passage and NO diffusion. NO itself can alter the function of gap junctions at the MEJ to affect the spread of vasodilation and constriction and change myogenic tone [79].

3.1 The Role of Connexins at the MEJ

Within the MEJ, there are gap junctions located at the points of cell-cell contact [2, 80]. The channels spanning these gap junctions are composed of two connexin hemichannels which connect across the intercellular space [81]. These channels allow for the movement of second messengers between cells, and they also propagate the flow of electrical signals [3, 82–84]. It is known that the vasculature contains at least four connexin isoforms: Cx37, Cx40, Cx43, and Cx45 [76, 85, 86], but thus far only Cx37, Cx40 and Cx43 have been identified within the MEJ [87, 88]. These connexins have been shown to associate with caveolin-1 in cultured endothelial cells, and when caveolin expression is lost, the expression of connexins is reduced in aortic and mesenteric arteries [89]. Additionally, through pulldown studies, eNOS has been shown to interact with both Cx40 and Cx37. In cell culture, knockdown of Cx37 leads to increased eNOS activity, suggesting that Cx37 may be a negative regulator of the enzyme [90]. Cx40^{-/-} mice have a decreased eNOS expression in their aortas, despite the fact that the mRNA levels remain the same, suggesting that connexins may potentially play a role in stabilizing eNOS [91].

It is not simply a direct interaction between eNOS and connexins which allows NO to have a regulatory role at the MEJ. It has been shown both *in vivo* and *in vitro* that NO can alter gap junction coupling. In human umbilical endothelial cells (HUVECs), NO donors enhanced connexin coupling. It was shown that this increase in coupling was due to a change in Cx40; NO increased the trafficking of Cx40 to the membrane in HeLa cells transfected with various connexin isoforms, suggesting that NO regulates Cx40 translocation and function [92]. NO can also affect connexin function via the reversible attachment of a nitroso group to a sulfur atom of an amino acid, termed S-nitrosation. One study found that eNOS localized to the MEJ was basally phosphorylated at Ser633 and Ser1177, both activating sites. This basal amount of activation led to localized production of NO at the MEJ, allowing for S-nitrosation of Cx43 at cysteine 271. This constitutive S-nitrosation enhanced permeability of the gap junction channel, but was altered with phenylephrine stimulation [74]. When HeLa cells transfected with Cx37 were mechanically stimulated to produce a rise in intracellular calcium, treatment with NO donor SNAP significantly decreased the number of cells responding, suggesting that NO was somehow reducing the permeability of Cx37 channels [93]. However, studies showing these specific roles for connexins within the vasculature vary depending on species, cell type, preparation and the presence or absence of other proteins. This suggests that connexin regulation of gap junction permeability is highly regulated and dependent on not only eNOS activity and NO signaling, but the presence of other connexins and proteins located within the microenvironment. Therefore, further investigation is required to fully understand the role of connexins in regulating NO signaling.

3.2 Hemoglobin α as a regulator of NO signaling at the MEJ

While NO quickly diffuses away from eNOS to reach its targets, there appears to be a system in place to regulate the amount of NO capable of traversing the cell membrane as models of unregulated diffusion do not consistently account for the biochemistry of eNOS-dependent signaling [6, 94]. Work by Straub et al. showed that hemoglobin α (Hb α) is present at the MEJ and acts to regulate NO passage. Hb α was identified during a proteomic analysis of the MEJ, and further investigation in vascular cell co-culture models (VCCC) confirmed its existence in ECs with enrichment in the MEJ fraction. Loss of Hb α in isolated thoracodorsal arteries using siRNA led to an increased Ach response and decreased constriction to phenylephrine. Within these isolated arteries and the VCCC, loss of Hb α resulted in increased NO diffusion, suggesting that this monomeric protein is responsible for regulating NO diffusion [95]. More recent evidence has demonstrated that coupling of Hb α with eNOS through a protein-protein interaction is critical for efficient NO scavenging [96].

It is well known that hemoglobin in red blood cells is an NO scavenger [97], but this work was the first to suggest that Hb α can also regulate NO within the endothelium itself. Ultimately, it is the oxidation state of the heme iron that controls whether Hb α will act to scavenge NO or permit its passage. When the heme iron is in the reduced state (Fe²⁺), it quickly reacts with the NO, limiting its passage. However, when the iron switches to its oxidized state (Fe³⁺), the reaction of the heme with NO is slow, allowing the molecule to make its way into the smooth muscle where it controls vasodilation and tone. Cytochrome B5 reductase 3 was found to be responsible for reducing Hb α heme iron, and both these proteins were shown to be in complex with eNOS [95]. Thus, NO signaling is not only controlled by the protein channels spanning the MEJ, but also by a protein complex located in close proximity to the source of NO.

4. NO and its role in vascular tone and reactivity

4.1 NO and vasodilation in the microcirculation

It is known that NO plays a lesser role in acetylcholine-induced vasodilation in the resistance arteries as compared to conduit arteries [36, 98–100], however this apparent difference cannot be explained by the lack of eNOS expression in the resistance arteries compared to conduits [101, 102]. Rather, it is well established that endothelial derived hyperpolarizing factor (EDHF) is the major vasodilator in the microcirculation [98]. Induction of EDHF depends on the activation of intermediate and small conductive Ca²⁺-activated K⁺ channels (IK_{ca}, SK_{ca}) on endothelial cells, which causes smooth muscle hyperpolarization and subsequent relaxation [103]. A plausible explanation for the switch from NO to EDHF based signaling is the phenomena of NO scavenging by hemoglobin α in the endothelium of resistance arteries as described above. Furthermore NO may signal independently of cGMP to cause vascular smooth muscle hyperpolarization [94]. Interestingly, studies have shown that S-nitrosothiols could also be a contributing factor to the EDHF response through endothelial IK_{ca} and SK_{ca} channel activation [104]. It is possible that pre-formed pools of S-nitrosothiols are compartmentalized, particularly at the MEJ where IK_{ca} and SK_{ca} channels are abundant, in order to stimulate the EDHF response. This would be inline with previous work demonstrating enrichment of eNOS at the MEJ,

providing a compartmentalized source for generating new S-nitrosothiols [74]. Further work is required to define specific targets of S-nitrosothiols and how they may contribute to the EDHF response.

4.2 NO as a Negative Feedback Regulator in Vasoconstriction

NO is traditionally thought of as a vasodilator, but through sensitive cross-talk between smooth muscle cells and endothelial cells, mediated in part by the MEJ, it can also act to modulate vasoconstriction. This phenomenon was first identified in the hamster cheek pouch arterioles in response to phenylephrine. Using Fluo-3 as a calcium indicator loaded into the cells of the arterioles, it was discovered that phenylephrine first induced a rise in calcium in the smooth muscle and that increase traveled to the endothelial cell. This increase in endothelial cell intracellular calcium led to NO production that fed back and acted to limit constriction as the addition of L-NAME, a NOS blocker, enhanced the vasoconstriction in response to phenylephrine [82]. This was initially somewhat debated, with the idea proposed that phenylephrine could be acting on adrenergic receptors on the endothelial cells themselves instead [105]. However, further research supports the original finding that a signal originating in the smooth muscle cell travels to the endothelium with adrenergic receptor stimulation [106].

Because MEJ communication is limited, bulk diffusion of enough calcium to activate eNOS is unlikely [107]. Instead, a more refined mechanism was sought to explain this NO modulation of vasoconstriction. All three isoforms of the IP₃ receptor have been identified in mesenteric arteries, with IP₃R1 and IP₃R2 having high expression levels in endothelial cells [108, 109]. Binding of IP₃ to these receptors promotes calcium channel opening, thereby raising intracellular calcium concentration [110]. Therefore, IP₃ could move from smooth muscle to endothelium to stimulate calcium release and NO production. Indeed, it has been shown that inhibition of IP₃ generation within smooth muscle cells attenuates vasoconstrictor-evoked increases in endothelium calcium levels within the intact rat mesenteric artery [107, 111].

Nausch et al. examined this idea by observing calcium pulsars, an IP₃ mediated calcium release event characterized by the Nelson lab, in third order mesenteric arteries from mice. At baseline, these events were observed within holes in the internal elastic lamina, but electrical field stimulation increased the frequency of these events about twofold for approximately 20 seconds after stimulation. This timing is consistent with calcium signaling observed within the smooth muscle cells, and the majority of the pulsars were located within the holes of the elastic lamina. Blocking smooth muscle global calcium rise did not hinder these events, suggesting a role for local IP₃ signaling [112]. Specifically, the KCa3.1 channel seems to be involved in the IP₃ mediated release of calcium as it has been shown to be localized to the MEJ [3, 113, 114] and blocking these channels inhibits myoendothelial feedback [7, 114]. However, some of these experiments suggest NO is not the mediating factor, but instead EDHF mediates the relaxation [112]. This discrepancy may be due to species or vascular bed differences as well as differing levels of expression in certain proteins such as hemoglobin α .

eNOS phosphorylation status has been examined after phenylephrine application to determine if there is a correlation in activation and NO feedback. Interestingly, in mouse mesenteric arteries, phosphorylation at Ser1179 (corresponding to Ser1177 in bovine and human) did increase with phenylephrine treatment, but the greatest amount of phosphorylation was seen at the point of maximal constriction [115]. These results lend support to the idea that eNOS phosphorylation may not be the final “on switch” for enzyme activity but instead acts as a permissive event, allowing other factors to ultimately increase activity.

This area of endothelial feedback in vasoconstriction still has many questions to be answered. While most work focuses on feedback with adrenergic receptor stimulation, the endothelial cells may modulate constriction in response to other vasoactive agents. Recent work has shown that a peptide deemed “vasoconstriction inhibiting factor” modulates angiotensin II mediated vasoconstriction by acting at the AT2 receptor [116]. Perhaps the MEJ and NO signaling may play some role in mediating this process or others. Conversely, the endothelial cells may also act to promote constriction with evidence for endothelium-derived contracting factors adding considerable complexity to the cross-talk story [117, 118].

4.3 Compartmentalized signaling and negative feedback

Thomas et al. propose five distinct concentration levels of NO activity: cGMP-mediated processes ($<1\text{--}30\text{nM}$), Akt phosphorylation ($30\text{--}100\text{nM}$), stabilization of HIF-1 α ($100\text{--}300\text{nM}$), phosphorylation of p53 ($>400\text{nM}$), and nitrosative stress ($1\text{ }\mu\text{M}$) [119]. The lower tiers allow for cell survival and normal physiological processes but the upper tiers lead to cell cycle arrest, apoptosis, and senescence [119]. However, while global increases in NO can lead to indiscriminate amounts of reactive nitrogen species signaling, a certain level is required for cellular signaling [120]. This argues for the importance of compartmentalization in NO production and reaction.

For eNOS derived NO, compartmentalization can be achieved by the activation of different spatially distinct populations of the enzyme. Because eNOS traffics back and forth to the plasma membrane, two distinct populations of eNOS exist: one at the plasma membrane and one at the Golgi apparatus [121]. These two populations have different thresholds of activation and rates of production [122, 123]. The enzyme which produces NO may also influence what effects it has. In cardiac myocytes, gene deletion of nNOS or eNOS produces isoform-specific phenotypes [124]. While eNOS is credited with most of the action of NO within the vasculature, nNOS blockade does cause systemic hypertension [125]. With the innervation of the vasculature by some nitrenergic nerves, some NO from nNOS is available to stimulate sGC and act at other points of the vessel wall [126]. Furthermore, blockade of nNOS leads to a reduction in basal blood flow in the human forearm and coronary circulation [127]. These two features of NO production may provide answers as to why not all populations of NO are bioequivalent.

One of the ways in which this localized production of NO can affect the function of the endothelial cell is through protein S-nitrosation. Because NO levels are higher around NOSs, a protein's chances of becoming S-nitrosated are much higher the closer it resides to

a NOS [128, 129]. Other factors influencing the chances for nitrosation include the characteristics of the protein's cysteines and the rate of denitrosation [128]. The structural characteristics that increase the likelihood of modification are not precisely known, but such factors as surrounding charge, conformation, and proximity to the surface could play a role [130].

S-nitrosation provides not only a way in which NO can regulate downstream signaling, but also a form of autoregulation for eNOS. eNOS appears to be the source of NO required for its own S-nitrosation which leads to enzyme inhibition [131]. eNOS derived NO can also modify the proteins responsible for eNOS activation and stabilization. For example, hsp90 becomes S-nitrosated on a cysteine residue located within the interacting region with eNOS, rendering its facilitating role to eNOS null [132].

5 Conclusions

NO is a powerful signaling molecule in resistance arteries with extensive roles in the maintenance of vascular tone and signaling. Through highly controlled production and diffusion, NO exerts varying signaling consequences depending on the cellular requirements. This one messenger offers a variety of responses, proving the importance of signaling microdomains and compartmentalization of second messenger production. Future directions focused on further refinement of NO signaling in resistance arteries and at the MEJ will be important in order to fully appreciate how such a simple molecule regulates the complex communication to control local blood flow and peripheral vascular resistance. Furthermore, additional studies will be needed to better understand how dysfunctional NO signaling in resistance arteries and the MEJ contributes to disease manifestation such as hypertension and peripheral artery disease. Because of these subtle differences in production leading to such different physiological outcomes, care needs to be taken when thinking of using NO as a therapy. Whereas NO production occurs in a highly regulated fashion in endothelial cells, NO donors simply deliver an unregulated dose to the entire vasculature. This could lead to unintended consequences and high variability in patient outcome.

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Highlights

- Nitric oxide regulates many biological functions in resistance arteries
- Resistance arteries house and compartmentalize multiple proteins involved in the production and transduction of nitric oxide allowing for efficient movement of the molecule to regulate vascular tone and reactivity

Figure 1. eNOS Binding Partners and alpha globin regulation of NO in a resistance artery
 Schematic on right shows an endothelial cell where (A) eNOS is kept inactive within caveolae by its association with caveolin-1, (B) another membrane protein, endoglin, promotes the activation of eNOS by increasing its association with HSP90 and helping to prevent its degradation, (C) calcium is required for eNOS activation, and it exerts its influence via the calcium modulated protein calmodulin; this protein reversibly binds to eNOS, facilitating the transfer of electrons from the reductase domain to the oxygenase domain, (D) NOSTRIN facilitates the movement of eNOS via caveolar endosomes, (E) NOSIP interacts with eNOS to facilitate movement along the actin skeleton; eNOS may move to a more perinuclear location due to its interactions with NOSIP. The bottom schematic shows an MEJ where intracellular communication is facilitated by gap junction proteins connexin 43 and 40 (A). Caveolae are localized to the MEJ allowing for caveolin-1-eNOS interaction. NO diffusion is controlled by α globin heme iron redox state which is modulated via Cyb5R3. When α globin is reduced (B), NO is scavenged, reducing its diffusion capacity to vascular smooth muscle. Alternatively, when α globin is oxidized, NO diffusion to vascular smooth muscle is increased (C).