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Review Article

Subcellular and cellular locations of nitric oxide synthase isoforms as determinants of health and disease

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ABSTRACT

The effects of nitric oxide in biological systems depend on its steady-state concentration and where it is being produced. The organ where nitric oxide is produced is relevant, and within the organ, which types of cells are actually contributing to this production seem to play a major determinant of its effect. Subcellular compartmentalization of specific nitric oxide synthase enzymes has been shown to play a major role in health and disease. Pathophysiological conditions affect the cellular expression and localization of nitric oxide synthases, which in turn alter organ cross talk. In this study, we describe the compartmentalization of nitric oxide in organs, cells, and subcellular organelles and how its localization relates to several relevant clinical conditions. Understanding the complexity of the compartmentalization of nitric oxide production and the implications of this compartmentalization in terms of cellular targets and downstream effects will eventually contribute toward the development of better strategies for treating or preventing pathological events associated with the increase, inhibition, or mislocalization of nitric oxide production.

Contents

Abbreviations: COPD, chronic obstructive pulmonary disease; DMD, Duchene muscular dystrophy; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NOS, nitric oxide synthase; NOS1, neuronal nitric oxide synthase; NOS2, inducible nitric oxide synthase; NOS3, endothelial nitric oxide synthase; NOSIP, nitric oxide synthase-interacting protein; RVLM, rostral ventrolateral medulla; sGC, soluble guanylyl cyclase.

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Introduction

Since 1987, when the endothelium-derived relaxing factor was identified as nitric oxide [1,2], numerous reports have indicated that this small gaseous molecule, nitric oxide, is a ubiquitous mediator of many different biological processes, such as vasodilation [3], neurotransmission [4,5], macrophage-mediated cytotoxicity [6], gastrointestinal smooth muscle relaxation [5], and bronchodilation [7], through a variety of downstream pathways (Fig. 1). According to Fick's laws of diffusion, the diffusion coefficient of nitric oxide is 4.8×10^{-5} cm^2 in water at 37 °C [8,9], similar to that of oxygen under comparable conditions [10]. It has been estimated that the half-life of nitric oxide varies from about 1 s in blood-free perfused guinea pig heart to 30 s in physiological buffers [8]. Based on these half-life values, the diffusion distances are expected to be in the 120–700 μm range [8]. Nitric oxide has been detected at a distance of 100 to 500 μm from RAW 264.7 macrophages stimulated with interferon, yielding a diffusion radius of 10 to 50 cells (assuming an average macrophage diameter of 10 μm) [11]. Thus, theoretically, based on its diffusion coefficient and the assumption that cells in culture are a representative model for the diffusion of nitric oxide in vivo, the effects of this gaseous molecule could extend to many cells beyond its production site.

However, estimations of the half-life of nitric oxide based on its diffusion radius do not apply to complex biological systems. Factors that limit nitric oxide diffusion and therefore its half-life in biological systems include its interactions with soluble guanylyl cyclase and other proteins (e.g., hemoglobin), lipids, and free radicals [11–13]. When measured in isolated rat aorta, for example, its diffusion radius was shown to be fourfold smaller in an aortic wall than in a homogeneous medium such as water [14]. It has also recently been reported that the cholesterol content in membranes decreases nitric oxide diffusion by 20 to 40% [12]. This decrease was attributed to changes in membrane fluidity caused by cholesterol. Nitric oxide efflux produced by activated macrophages was also reduced by 41% in the presence of albumin and by 53 to 70% in the presence of liposomes, indicating that intracellular structures or biomolecules could also limit nitric oxide diffusion [11], thereby establishing compartmentalized effects of nitric oxide within the cells.

Depending on the environment, other factors can affect the half-life of nitric oxide and therefore its diffusion. When high concentrations of nitric oxide are produced by activated inducible nitric oxide synthase (NOS2)[1], and superoxide anion is present, the formation of peroxynitrite will limit the diffusion of nitric oxide [8]. In addition, depending on the oxygen gradient near mitochondria, cytochrome *c* oxidase can become a target of nitric oxide, resulting in the inhibition of mitochondrial respiration [13].

In addition to the diffusion of nitric oxide from its production site, the partitioning of nitric oxide between polar and apolar media could play a major role in terms of localized effects [15]. Nitric oxide and oxygen have similar partition coefficients in apolar media, being 70 times more soluble in hydrophobic than in hydrophilic media [16]. Therefore, both molecules are more concentrated in a hydrophobic milieu, such as liposomes, lipoproteins, or biomembranes or within the hydrophobic pockets of proteins, than in polar-based environments [16,17]. Higher concentrations of nitric oxide and oxygen in an

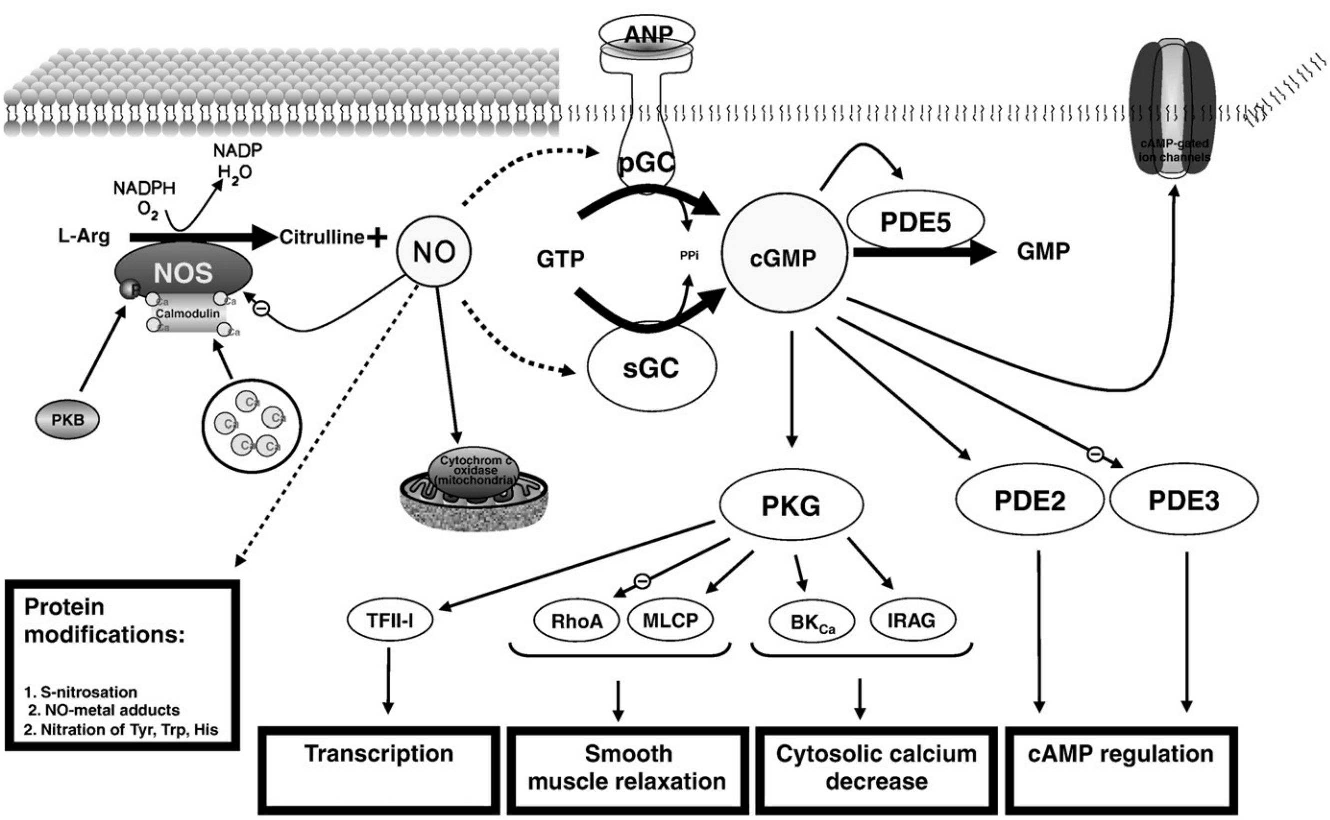

Fig. 1. Nitric oxide generation and signaling. Nitric oxide, generated by NOS, activates soluble guanylate cyclase (sGC) and particulate guanylate cyclase (pGC) and inhibits cytochrome *c* oxidase. cGMP activates cGMP-dependent protein kinases (PKG). As shown, some downstream pathways and cellular functions (boxes) are involved in the effects of endogenous cGMP. The concentration of cGMP can be controlled by the action of phosphodiesterases (PDE). In addition, nitric oxide can affect other pathways through protein modifications (nitric oxide–metal adduct formation, S-nitrosation, nitration). For instance, the nitration of specific tyrosine residues in the β-subunit of Complex V results in lower ATPase activity during nitrative stress or aging [137,138]. ANP, atrial natriuretic peptide; PK, protein kinases (letter indicates the type of kinase); IRAG, IP_3 receptor-associated cGKIβ substrate; MLCP, MLC phosphatase; RhoA, a substrate for PKG; BK_{Ca}, large-conductance Ca^{2+}-activated K^+ channels. Other details were previously described by Hofmann et al. [139].