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Review

S-Nitrosylation in neurogenesis and neuronal development ☆

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ABSTRACT

Background: Nitric oxide (NO) is a pleiotropic messenger molecule. The multidimensional actions of NO species are, in part, mediated by their redox nature. Oxidative posttranslational modification of cysteine residues to regulate protein function, termed S-nitrosylation, constitutes a major form of redox-based signaling by NO.
Scope of review: S-Nitrosylation directly modifies a number of cytoplasmic and nuclear proteins in neurons. S-Nitrosylation modulates neuronal development by reaction with specific proteins, including the transcription factor MEF2. This review focuses on the impact of S-nitrosylation on neurogenesis and neuronal development.
Major conclusions: Functional characterization of S-nitrosylated proteins that regulate neuronal development represents a rapidly emerging field. Recent studies reveal that S-nitrosylation-mediated redox signaling plays an important role in several biological processes essential for neuronal differentiation and maturation.
General significance: Investigation of S-nitrosylation in the nervous system has elucidated new molecular and cellular mechanisms for neuronal development. S-Nitrosylated proteins in signaling networks modulate key events in brain development. Dysregulation of this redox-signaling pathway may contribute to neurodevelopmental disabilities such as autism spectrum disorder (ASD). Thus, further elucidation of the involvement of S-nitrosylation in brain development may offer potential therapeutic avenues for neurodevelopmental disorders.
This article is part of a Special Issue entitled Redox regulation of differentiation and de-differentiation.

1. Redox signaling by S-nitrosylation

NO was first identified as an Endothelium Derived Relaxing Factor (EDRF) [1], but also serves as a more widespread signaling molecule [2]. NO-mediated physiological processes include vasodilation, immune function, neurotransmission, and neuronal maturation [2–6]. Three types of NO synthases generate endogenous NO in mammalian cells: neuronal NOS (nNOS, NOS1); inducible NOS (iNOS, NOS2); and endothelial NOS (eNOS, NOS3) [2,7,8]. All three NOS isoforms are expressed in the brain [9], but differential expression of the three isoforms with different characteristics orchestrates the diverse range of NO-mediated biochemical reactions [2]. However, excessive or sustained production of NO and NO-derived reactive nitrogen species (RNS) contributes to pathological consequences, including tissue damage, carcinomas, inflammatory conditions, and neurodegenerative diseases [3,4]. Initial studies found that NO binds to the heme moiety of guanylate cyclase and induces its active conformation, resulting in the formation of cyclic GMP to induce vasodilation [2,5]. Subsequently, studies elucidated that S-nitrosylation, an oxidative modification of cysteine thiol by NO-related species, affects protein activity and stability, protein–protein interactions, and protein trafficking and location. In many ways, S-nitrosylation serves as a post-translational modification reminiscent of phosphorylation or acetylation, and, in fact, may be even more ubiquitous [2,5]. The list of target proteins and physiological and pathological events evoked by S-nitrosylation is continually lengthening. S-Nitrosylation has emerged as a major mechanism for the signaling actions of NO.

Concerning the S-nitrosylation reaction, the d-orbitals of the sulfur atom at the thiol core confer high reactivity and chemical plasticity, allowing for multiple oxidation states (Fig. 1A), and are the main target of RNS for the formation of reversible S-nitrosothiols [5]. In some cases, e.g., matrix metalloproteinase-9 (MMP-9), S-nitrosylation subserves further irreversible oxidation, leading to sulfonation ($-SO_3H$) of the critical cysteine thiol [10]. S-Nitrosylation may be mediated, at least in part, by transnitrosylation reactions between proteins that transfer the NO group from the thiol of one protein to the thiol of another (Fig. 1B) [11–13]. In contrast, several denitrosylases have been reported to remove the NO group from S-nitrosylated thiol side chains [2,14]. Thus, the balance between nitrosylation and denitrosylation tightly regulates the specific status of S-nitrosylation on cellular proteins. These regulatory mechanisms of S-nitrosylation offer control of many aspects of cellular physiology [2,5]. Recent reviews have discussed the importance of S-nitrosylation on normal cellular physiology [5] as well as pathological

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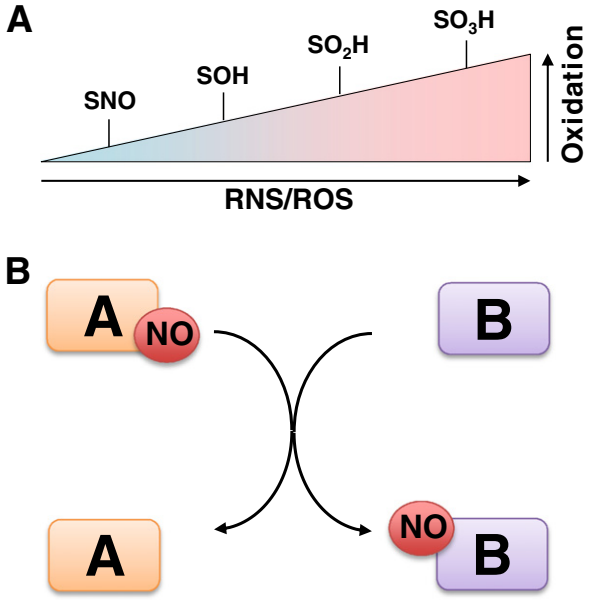

Fig. 1. Progressive posttranslational oxidation of cysteine thiols and mechanism of transnitrosylation. A) Reactive nitrogen species (RNS) and/or reactive oxygen species (ROS) oxidize redox sensitive-cysteine thiols [5]. For some protein cysteine residues, as RNS/ROS levels increase, the oxidation status of the thiol may progress from S-nitrosothiol (–SNO), to sulfenic acid (–SOH) to sulfinic acid ($–SO_2H$), and finally to a irreversible sulfonic acid ($–SO_3H$) (as occurs for MMP-9) [10]. B) Transfer of a nitric oxide (NO) group from one protein to another (transnitrosylation) represents an enzymatic reaction mechanism for effecting nitrosation. The interaction of SNO-protein A with protein B determines the specificity of transnitrosylation. Since the NO group is transferred from SNO-protein A to a specific cysteine thiol on protein B, protein A is regarded as a nitrosylase and protein B as a denitrosylase (since protein A surrenders its NO group to protein B).

states, particularly in the nervous system [2]. Here, we will focus on the role of S-nitrosylation in neurogenesis and neuronal development.

2. CREB (cyclic-AMP response element binding protein)-dependent dendritic growth and nitrosylation pathways

Dendrites are branched extensions from neurons that receive afferent inputs. The molecular and cellular mechanisms underlying dendritic development have been a focus of research for the past several decades. Recent studies revealed that dendritic development is regulated in both a neuronal activity-dependent and -independent manner [15,16]. Both signaling pathways ultimately lead to activation of transcription factors such as the nuclear effector CREB (cAMP response element-binding protein). CREB belongs to a family of transcription factors with a highly conserved basic region/leucine zipper domain. CREB was originally described as a cellular transcription factor that binds the cAMP-response element (CRE) [17]. CREB mediates calcium-dependent gene expression induced by depolarization in neuronal cells [18]. To date, CREB is arguably the best studied transcription factor in the context of the nervous system.

The activation of CREB is triggered by a wide variety of signaling processes in neurons. During dendritic growth, neuronal activity evokes calcium-dependent signaling and activates CREB kinases, causing phosphorylation of CREB serine-133, which results in CREB activation [19,20]. In contrast, activity-independent signaling is elicited by extracellular factors such as neurotrophins. The neurotrophins encompass a family of closely related peptides, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and NT-4 [21,22]. Among them, BDNF is the most extensively studied molecule involved in dendritic development. BDNF induces NO generation and increases CREB binding activity an in nNOS-dependent and serine-133 phosphorylation independent manner [23,24]. Interestingly, S-nitrosylation affects BDNF/CREB-dependent dendrite outgrowth at multiple levels of control, as described below [24–28].

One key action of BDNF concerns its affects on the localization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and how this affects S-nitrosylation-mediated signaling. GAPDH is generally considered to be a housekeeping enzyme in the glycolysis cascade. However, GAPDH also acts as a signal mediator between the cytoplasm and the nucleus (despite its lack of a nuclear localization signal, NLS) [2,29–31]. Importantly, the shuttling of GAPDH between the cytoplasm and nucleus is mediated by S-nitrosylation at the catalytic cysteine-150 (forming SNO-GAPDH) [25]. This S-nitrosylation reaction abolishes GAPDH enzymatic activity, but promotes its delivery to Siah (seven in absentia homolog 1, a ubiquitin E3 ligase) [25] (Fig. 2). Since Siah harbors an NLS, the SNO-GAPDH/Siah complex translocates to the nucleus [25]. Notably, this SNO-GAPDH translocation is negatively regulated by a second S-nitrosylation mechanism as follows. Sen et al. performed a yeast two-hybrid screen for proteins interacting with GAPDH and identified a cytoplasmic 52 kDa protein enriched in the brain, termed GOSPEL (GAPDH's competitor of Siah protein enhancer life) [26]. Under physiological conditions or low nitrosative stress, GOSPEL physically interacts with GAPDH in an S-nitrosylation-dependent manner. SNO-GOSPEL at cysteine-47 competes with Siah for binding to GAPDH, thereby retaining GAPDH in the cytoplasm [26] (Fig. 2).

The nuclear SNO-GAPDH/Siah complex enhances CREB-mediated dendrite outgrowth through at least two epigenetic chromatin-remodeling regulatory mechanisms. In the nucleus, SNO-GAPDH stabilizes Siah, which facilities ubiquitination and degradation of nuclear proteins such as SUV38H1 [28]. SUV38H1 is a major histone methylating enzyme that trimethylates lysine 9 on histone H3 (H3K9), a molecular signature of gene silencing. In neurons, BDNF treatment induces the nuclear translocation of SNO-GAPDH/Siah and ubiquitin-mediated degradation of SUV38H1. This decrease in histone H3K9 trimethylation allows enhanced CREB binding [28] (Fig. 2). In addition, SNO-GAPDH propagates NO signaling in the nucleus by transnitrosylation [27]. Since the NO group is transferred from the donor's SNO-thiol to the acceptor's thiol, donor proteins are regarded as transnitrosylases and acceptor proteins as denitrosylases (Fig. 1B). Recent studies have demonstrated transnitrosylation reactions in multiple biological systems [2,14], e.g. SNO-hemoglobin and anion exchanger 1 [11], SNO-thioredoxin and caspase-3 [12,32], SNO-caspase-3 and X-linked inhibitor of apoptosis, and SNO-cyclin-dependent kinase 5 and dynamin related protein 1 [33].

Nuclear translocated SNO-GAPDH can also serve as a transnitrosylase [27]. SNO-GAPDH interacts with nuclear proteins, including sirtus-1, DNA-activated protein kinase, B23, and histone deacetylase 2 (HDAC2), in an S-nitrosylation dependent fashion [27,34]. The transnitrosylation reaction can confer selectivity by targeted transfer of an NO group to reactive cysteine residues of specific substrates [27]. In the case of HDAC2, it is S-nitrosylated from SNO-GAPDH by transnitrosylation, and this reaction is also involved in BDNF-induced dendritic outgrowth [24] (Fig. 2). Under basal conditions, HDAC2 is associated with promoters of CREB target genes. BDNF stimulation induces S-nitrosylation of cysteine-262 and -274 on HDAC2 causing dissociation from these promoters. The dissociation induces rapid acetylation of histones H3 and H4, and expression of CREB target genes, leading to dendritic growth. These findings show that nitrosylation of HDAC2 promotes epigenetic changes and activation of CREB-dependent genes that are essential for dendritic development [24] (Fig. 2). Taken together, these experiments suggest that diverse S-nitrosylation signaling pathways convey extracellular cues to the nucleus and control the activity of two key chromatin modifiers (SUV38H1 and HDAC2) to influence dendritic outgrowth. Importantly, chromatin remodeling or epigenetic alteration acts as an interface between genetic risk and environmental factors, and, as such, is an evolving biological mechanism for gene × environment (GxE) interactions in the pathogenesis of an array of neurodevelopmental disorders, including schizophrenia and autism [35–38]. Any disturbance in the epigenetic remodeling