Say NO to Neurodegeneration: Role of S-Nitrosylation in Neurodegenerative Disorders

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Key Words

S-nitrosylation • Neurodegeneration • Nitric oxide • Neurodegenerative disorders

Abstract

Nitric oxide (NO) is an important signaling molecule that controls a wide range of biological processes. One of the signaling mechanisms of NO is through the S-nitrosylation of cysteine residues on proteins. S-nitrosylation is now regarded as an important redox signaling mechanism in the regulation of different cellular and physiological functions. However, deregulation of S-nitrosylation has also been linked to various human diseases such as neurodegenerative disorders. Nitrosative stress has long been considered as a major mediator in the development of neurodegeneration, but the molecular mechanism of how NO can contribute to neurodegeneration is not completely clear. Early studies suggested that nitration of proteins, which can induce protein aggregation might contribute to the neurodegenerative process. However, several recent studies suggest that S-nitrosylation of proteins that are important for neuronal survival contributes substantially in the development of various neurodegenerative disorders. Thus, in-depth understanding of the mechanism of neurodegeneration in relation to S-nitrosylation will be critical for the development of therapeutic treatment against these neurodegenerative diseases.

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Introduction

Nitric oxide (NO) is a well-known signaling molecule that induces endothelium relaxation in response to a variety of stimuli. In mammals, NO is synthesized by nitric oxide synthases (NOS) through the conversion of L-arginine to NO and L-citrulline. The discovery of NO as an endothelium-derived relaxing factor (EDRF) was more than two decades ago, and since then, increasing numbers of pathways have been shown to be modulated by NO. However, imbalance of NO signaling has also been linked to different human diseases. In particular, increasing evidence suggests that S-nitrosylation is involved in the pathogenic process of various neurodegenerative disorders. Therefore, a thorough understanding of how NO modulates different physiological and cellular pathways will identify potential new pharmacological targets for neurodegeneration in the future.

How NO Mediates Signal Transduction

Initial work showed that NO mediates its effect by activating guanylate cyclases (GC) through the binding of NO to the heme group of GC [1]. This binding induces a conformational change and activates the enzyme for the production of cGMP [1]. Increased level of cGMP then activates selected pathways to induce cellular responses

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1424–862X/06/0156–0307\$23.50/0

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such as vasodilation [1]. Aside from the heme group, NO also reacts with cysteine residues on target proteins to form nitrosothiols that mediate signal transduction. S-nitrosylation is a reversible process that is facilitated by an acid-base amino acid motif within the protein structure [2, 3]. This characteristic motif was discovered by alignment of proteins shown to be S-nitrosylated by NO [3]. An example of this motif is found on the beta subunit of hemoglobin in which the Cys93 (His92-Cys93-Asp94) is known to be modified by NO [3]. However, an important feature of the acid-base motif is that it is not restricted to the primary structure of the protein sequence. For example, cysteine residues 221 and 273 of dimethylargininase-1 (DDAH-1) are modified by NO through S-nitrosylation, but these cysteines (Asn220-Cys221-Ile222 and Thr272-Cys273-Cys274) are not flanked by acidic or basic amino acids on the primary sequence [4]. Instead, these cysteine residues (Cys221 and Cys273) are surrounded by acidic and basic amino acids that are brought together through structural folding [4].

Although attempts have been made to elucidate how proteins are being S-nitrosylated, the exact mechanism is not clear. It is hypothesized that the transfer of NO group between nitrosothiols is the major route of how S-nitrosylation signaling is being conducted. The most common reservoir of nitrosothiols is S-nitrosoglutathione (GSNO). GSNO is generated when NO reacts with oxygen to form N_2O_3 which subsequently reacts with glutathione to form the GSNO. Several enzymes have been identified to metabolize GSNO and to facilitate S-nitrosylation as the signaling mechanism for NO. For instance, gamma-glutamyl transpeptidase (GGT) converts GSNO to S-nitroso-cysteinyl glycine (CGSNO), which is a more potent NO donor in the process of S-nitrosylation [5]. Another enzyme, S-nitrosoglutathione reductase (GSNOR), releases NO from GSNO and functions as a scavenger system for nitrosothiols in the biological system [6]. How these enzymes coordinate to orchestrate the signaling of NO through S-nitrosylation is still not clear, but it is known that dysfunction of these enzymes affects some important physiological function [7–9].

How S-Nitrosylated Proteins Are Identified and Detected

Because of the labile nature of the modification, detection of S-nitrosylation in proteins remains to be a difficult task. Nitrosothiols can be detected by chemiluminescence, mass spectrometry, antibodies that recognize

nitrosothiols, colorimetric or fluorometric method, and the biotin switch assay [10]. Usually, a combination of these methods is used to confirm a protein that is being S-nitrosylated [10]. Two studies have reported high-throughput proteomic approaches to identify proteins that are S-nitrosylated based on the biotin-switch assay [11, 12]. Hao et al. [12] described their SNO-Site identification (SNOSID) approach for screening proteins that can be S-nitrosylated in brain tissue with the use of a combination of the biotin-switch assay and the liquid chromatography tandem mass spectrometry (LC-MS) analysis. Their approach involved treating the rat cerebellum homogenate with GSNO and then using the biotin switch assay to label proteins that were S-nitrosylated with biotin. This approach identified 6 endogenously S-nitrosylated proteins and 58 proteins that were modified after treatment with GSNO [12]. In a similar study, 18 S-nitrosylated proteins were identified in human aortic smooth muscle cells after exposure to S-nitrosocysteine or propylamine propylamine NONoate [11]. By comparing the two studies, only two proteins (microtubule-associated protein and 14-3-3) were identified in both reports [11, 12]. These findings suggest that an approach similar to SNOSID is not a very robust method in identifying S-nitrosylated proteins from tissues. Another interesting observation from these two reports is that the S-nitrosylated cysteine residues are mostly not flanked by the acid-base amino acid motif on the primary protein structures [11, 12], which suggests that the 3-dimensional acid-base motif might be more important in the process of S-nitrosylation.

Neuronal Processes That Are Modulated by S-Nitrosylation

Gene Transcription

Redox signaling is known to affect gene transcription, and NO is emerging as an important molecule that regulates gene expression in relation to redox environment. At least four well-known transcription factors are known to be modulated by NO through S-nitrosylation. NO has been shown to affect the activity of estrogen receptor, nuclear factor-kappa B (NF- κ B), hypoxia inducible factor (HIF) and cAMP response element binding (CREB) through S-nitrosylation [13–17]. In estrogen receptor and HIF, NO directly S-nitrosylates these transcriptional factors and modulates their ability to activate gene transcription [13, 16]. For NF- κ B and CREB, NO mainly affects their transcriptional activities indi-

rectly [14, 15, 17]. In the basal state, NF- κ B is bound to inhibitory κ B (I κ B) and is located at the cytoplasm. When the NF- κ B pathway is activated, I κ B kinase (IKK) phosphorylates I κ B and promotes I κ B degradation [14, 15]. Without the inhibition from I κ B, NF- κ B translocates to the nucleus and initiates gene transcription [14, 15]. NO inhibits NF- κ B activation indirectly by S-nitrosylation of IKK and prevents the phosphorylation of I κ B. This prevents the degradation of I κ B and inhibits the activation of NF- κ B pathway [14, 15]. CREB is an important transcriptional activator in neurotrophin signaling such as brain-derived neurotrophic factor (BDNF). A study suggests that the BDNF-induced activation of CREB is dependent on S-nitrosylation of components in the CREB DNA-binding complex [17]. However, which components are being S-nitrosylated during the BDNF-induced CREB transcriptional activation is not known [17].

Synaptic Transmission

NO is the first gaseous molecule that is considered as a neurotransmitter in the nervous system. The importance of NO in the synaptic transmission is best demonstrated by its effect on N-methyl D-aspartate receptor (NMDAR). S-nitrosylation of cysteine 399 of the NR2A subunit in the NMDAR inhibits its activity [18, 19]. This negative feed back mechanism is facilitated by the proximate location of nNOS which is linked to the NMDAR via the PDZ domain. Since nNOS is activated by Ca²⁺, influx of Ca²⁺ during the synaptic activation of NMDAR will increase the production of NO by nNOS. NO can modulate synaptic transmission at several levels. The NMDAR downstream signaling molecules such as p21Ras and Dexas are activated by S-nitrosylation [20–22]. In addition, S-nitrosylation of N-ethylmaleimide sensitive factor (NSF) promotes the surface expression of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPA) [23]. Though it is clear that the proper function of NMDAR transmission is indispensable with the NO, it remains to be determined how exactly NO can modulate the NMDAR synaptic transmission. Apart from modulating the NMDAR, NO also affects the transmission of G-protein-coupled receptor (GPCR) through S-nitrosylation. A recent study has shown that S-nitrosylation of GPCR kinase (GRK) attenuates GPCR agonist induced desensitization and internalization of β -adrenergic receptor [8]. Further studies are needed to see if NO can S-nitrosylate other GRK and can affect the signal transduction of other GPCRs.

Modulation of Physiological Function

NO's role as a signaling molecule was first demonstrated in the cardiovascular system as an EDRF. More recent studies suggest that NO functions at different levels to regulate the respiratory and cardiovascular systems in response to metabolic demands [24, 25]. In the vascular system, S-nitrosylation of β -globin Cys-93 in oxyhemoglobin has been reported as a major reservoir of nitrosothiol [24, 25]. Further studies proposed that in tissues with high metabolic demand, the release of O₂ from hemoglobin will simultaneously trigger the release of NO which induces local vasodilatation to increase blood supply in these active tissues [24, 25]. The importance of S-nitrosylation in the cardiovascular system signaling is further supported by the finding that nitrosothiols released from deoxygenated blood can induce hyperventilatory response via brain stem during hypoxic condition [26].

Nitric Oxide, S-Nitrosylation and Neurodegenerative Disorders

NO has long been regarded as a contributor in the process of neurodegeneration. Early studies suggested that nitrosative stress induced by inflammatory response is an important factor for the degeneration of neurons. This is evident by the fact that increased nitration of protein aggregates are prominent in various neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), synucleinopathies, and tauopathies [27, 28]. The increased nitration of proteins can be initiated by increased production of NO during the process of neuroinflammation, and generation of free radicals by dysfunctional mitochondria which are commonly observed in various neurodegenerative disorders. The combination of free radicals such as superoxide anion and NO will result in the formation of highly reactive peroxynitrite. Peroxynitrite can then nitrate the tyrosine residues to 3-nitrotyrosine on proteins, induce lipid peroxidation, and cause DNA damage [29]. However, some studies now suggest that NO can contribute to the pathogenic process of neurodegeneration through S-nitrosylation of different critical proteins that are important for the survival of neurons.

Excessive nitrosative stress has long been linked to pathogenesis of amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and Parkinson's disease (PD). The strongest evidence for the linkage of nitrosative stress and neurodegeneration is in the presence of ni-

trated protein aggregates in brain tissues of patients afflicted with these neurodegenerative disorders [27, 28]. This observation also suggests that an imbalance of S-nitrosylation of proteins due to increased nitrosative stress can contribute to the pathogenesis of various neurodegenerative diseases. This notion is supported by a recent report that depletion of S-nitrosothiols by mutated superoxide dismutase 1 (SOD1) contributes to the pathogenesis of ALS [30]. ALS is a motor neuron degenerative disease characterized by adult onset and degeneration of motor neurons in the spinal cord, brain stem and cortex [31]. The disorder is mostly sporadic, but mutations in SOD1 have been linked to rare familial forms of ALS [31]. Since the discovery that SOD1 mutations cause ALS, animal model of ALS has been set up by over-expression of mutant SOD1 in mice [31]. This animal model recapitulates some fundamental features of ALS in patients such as selective degeneration of motor neurons in the spinal cord, and has proven to be a valuable tool in understanding the pathogenic mechanism of ALS. The linkage of S-nitrosylation and the development of ALS was found in a recent study that selective depletion of nitrosothiols are prominent in the spinal cords of the SOD1 mutant over-expression animal model of ALS [30]. The mechanism of how the depletion of nitrosothiols can contribute to degeneration of motor neurons in ALS is not exactly known, but more detailed study suggests that it is related to the decrease levels of S-nitrosylated GAPDH [30]. Since S-nitrosylation of GAPDH is known to regulate its translocation to the nucleus and the process is critical for cell survival, it is hypothesized that decreased S-nitrosylation of GAPDH can contribute to the demise of motor neurons in ALS [30]. The important role of nitrosative stress in AD and PD is clearly demonstrated by the prominent deposition of nitrated protein aggregates in brain tissues of affected patients [27, 32]. The molecular mechanisms of how nitrosative stress contributes to AD and PD are not completely clear, but it is generally believed that nitration of protein can induce the formation of protein aggregates, which can ultimately cause the degeneration of neurons. Some recent findings in PD might provide the molecular mechanism of how nitrosative stress can contribute to the process of neurodegeneration.

Nitrosative Stress and PD

PD is a common degenerative disorder which is marked by movement impairments due to a progressive loss of tyrosine hydroxylase-positive dopaminergic neurons in the brain stem. This characteristic neuronal de-

generation is accompanied by the presence of intracellular protein aggregates designated as Lewy bodies (LB) which are enriched with ubiquitin and α -synuclein. The cause of PD is not clear, but from pathological studies, it is proposed that oxidative damage and protein aggregation is central to the pathogenesis of PD. Most PD cases are sporadic, but a small percentage of them are familial-linked. From genetic linkage studies, mutations in α -synuclein, LRRK2 and UCH-L1 cause the autosomal-dominant form of PD while mutations in PINK1, DJ-1 and parkin cause the autosomal-recessive form of PD [33]. Genetic and biochemical studies of these genes and their gene products have yielded new insights on how malfunctions of various pathways induce degeneration of dopaminergic neurons in PD. In agreement with the established hypothesis, genes linked to familial PD are related to mitochondria function (PINK1), handling of misfolded proteins (parkin and DJ-1) or itself is a protein that is prompt to misfolding (α -synuclein). If nitrosative stress is crucial in the development of PD, familial PD genes or pathways that are linked to these gene products should be sensitive to NO. Studies suggested that this is actually the case. The first evidence comes from the study that α -synuclein, which is the major component of LB, is specifically nitrated in LB [32]. On the other hand, at least two PD familial-linked gene products have been shown to be S-nitrosylated by NO. For instance, S-nitrosylation of Cys46 in DJ-1 has been shown to affect its natural dimerization tendency, but the physiological significance of this is not clear [34]. Another PD-linked gene product that can be S-nitrosylated is parkin [35, 36]. Mutations of parkin cause autosomal-recessive early onset parkinsonism, and biochemical studies have shown that parkin functions as an E3 ligase in the ubiquitin proteasomal system (UPS) [37]. Various studies have shown that parkin can protect neurons from different insults [38], and deletion of parkin in mouse has shown to affect the normal survival of monoaminergic neurons [39]. Parkin is a RING finger domain type E3 ligase, and one of the characteristics of this RING finger domain is the existence of considerable number of highly conserved cysteine residues within the domain. We initially suspected that these cysteine residues can be S-nitrosylated by NO, and this modification will affect the normal function of parkin. To test this hypothesis, we performed biotin-switch and fluorometric assays [10] that can detect S-nitrosylation under in vitro and in vivo conditions to determine if parkin can be modified by NO [35]. In agreement with our hypothesis, we and another group independently showed that parkin is modified by NO

through S-nitrosylation under different conditions [35, 36]. S-nitrosylation of parkin impairs its E3 ligase activity and compromises its protective function. The selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to mediate its toxicity through the production of oxidative free radicals such as peroxynitrite [40]. Our study also confirmed that S-nitrosylation of parkin is relevant to the pathogenesis of PD as increased parkin S-nitrosylation is prominent in mouse MPTP model of PD [35]. In post-mortem brain tissues from PD patients, increased levels of both nitrosothiols and S-nitrosylated parkin were found [35]. Taken together, these findings suggested that inhibition of parkin's function by S-nitrosylation can contribute to the pathogenesis in the more common form of sporadic PD [35, 36].

The hypothesis that nitrosative stress through S-nitrosylation is a major contributor in the development of PD is supported by several recent studies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a well-known enzyme that functions in the glycolysis pathway. However, a previous study showed that nuclear translocation of GAPDH is associated with a number of cell death stimuli. Interestingly in a recent study, it was shown that S-nitrosylation of GAPDH induces its nuclear translocation through the binding of an E3 ubiquitin ligase Siah1, and this translocation was found to initiate apoptosis in the cell [41]. In the mouse MPTP model of PD, increased S-nitrosylation of GAPDH is observed in the brain of MPTP-treated animals [42]. This increase in S-nitrosylation of GAPDH can be reversed by deprenyl, which is known to provide neuroprotection against MPTP induced toxicity [42]. These studies suggest that S-nitrosylation of GAPDH is directly linked to the pathogenesis of PD [42].

Another recent report that supports the role of nitrosative stress in the process of neurodegeneration is the inhibition of protein-disulphide isomerase (PDI) by NO through S-nitrosylation [43]. Misfolded proteins have long been suspected to aggravate the process of neurodegeneration and it has been proposed that nitration of proteins can cause protein misfolding which can consequently damage neurons [27]. This hypothesis is supported by studies showing that overexpression of heat shock protein 70 (HSP70), which prevent protein misfolding, can protect neurodegeneration in different animal models. Whether nitrosative stress can affect the function of HSP70 related protein is not clear, but a recent study found that a protein that handles protein misfolding (PDI) can be S-nitrosylated and this modifica-

Fig. 1. Schematic diagram illustrating how nitrosative stress can contribute to neurodegeneration through S-nitrosylation of different proteins in the cell. Increased nitrosative stress due to up-regulation of iNOS or nNOS activity can increase the levels of protein S-nitrosylation. S-nitrosylation of parkin and PDI inhibit their neuroprotective activities against protein misfolding and protein aggregation. Misfolded or aggregated proteins can impair the normal function of UPS and further exacerbate the process of neurodegeneration. Increased S-nitrosylation of GAPDH can promote apoptosis in neurons that have been made vulnerable because of the accumulation of misfolded or aggregated proteins.

tion is related to the pathogenesis of PD and AD [43]. PDI is an enzyme that assists the maturation and transport of unfolded proteins [43]. PDI prevents protein misfolding by assisting the proper formation of disulphide bonds in the process of protein folding [44]. The finding that S-nitrosylation of PDI can compromise its function provides another direct link between nitrosative stress and misfolded protein stress in the process of neurodegeneration. Furthermore, increased levels of PDI S-nitrosylation are observed in both PD and AD patients [43]. Taken together, these reports suggest that nitrosative stress

through S-nitrosylation is crucial in the process of neurodegeneration (fig. 1) and further research is needed in this area.

Concluding Remarks

Increasing evidence suggests that NO is an important contributor to neurodegeneration. In-depth understanding of how imbalance of NO metabolism can contribute

to neuronal cell death will be a crucial task in the future in order to formulate the possible neuroprotective strategies in the treatment of various neurodegenerative disorders.

Acknowledgements

KKKC is supported by grants from the Research Grants Council of Hong Kong (HKUST6435/06M, HIA05/06.SC04).

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