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Pathophysiological Role of S-Nitrosylation and Transnitrosylation Depending on S-Nitrosoglutathione Levels Regulated by S-Nitrosoglutathione Reductase

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Abstract

Nitric oxide (NO) mediates various physiological and pathological processes, including cell proliferation, differentiation, and inflammation. Protein S-nitrosylation (SNO), a NO-mediated reversible protein modification, leads to changes in the activity and function of target proteins. Recent findings on protein-protein transnitrosylation reactions (transfer of an NO group from one protein to another) have unveiled the mechanism of NO modulation of specific signaling pathways. The intracellular level of S-nitrosoglutathione (GSNO), a major reactive NO species, is controlled by GSNO reductase (GSNOR), a major regulator of NO/SNO signaling. Increasing number of GSNOR-related studies have shown the important role that denitrosylation plays in cellular NO/SNO homeostasis and human pathophysiology. This review introduces recent evidence of GSNO-mediated NO/SNO signaling depending on GSNOR expression or activity. In addition, the applicability of GSNOR as a target for drug therapy will be discussed in this review.

Key Words: Nitric Oxide, S-nitrosylation, Transnitrosylation, GSNO, GSNOR

INTRODUCTION

Nitric oxide (NO) is a free radical that is formed in numerous cell types, including endothelial, muscle, and neuronal cells. NO can play the role of a physiological or pathological effector depending on the target signaling pathway (NamKoong and Kim, 2010; Park *et al*., 2011; Eo *et al*., 2013; Ryu *et al*., 2015). There are three subtypes of NO synthases (NOSs) in mammalian organisms, namely neuronal NOS (nNOS) and endothelial NOS (eNOS), which are constitutive forms, and inductive NOS (iNOS). The biological action of NO is believed to be mediated mainly via guanylate cyclase activation and cyclic guanosine-3',5'-monophosphate (cGMP) production. However, S-nitrosylation, a covalent reaction of an NO group with a reactive cysteine thiol on target proteins, has emerged as another major mechanism for NO bioactivity (Hess *et al*., 2005). The formation of S-nitrosoproteins (i.e., protein-SNOs) generally regulates protein function by allosteric or direct modification of active site cysteine (Lipton *et al*., 1993; Hess *et al*., 2005). Some leading groups discovered and characterized this unique process on N-methyl-D-aspartate-type glutamate receptors (NMDARs) in the central nervous system (CNS) and demonstrated that NO inhibits excessive NMDAR activity via protein S-nitrosylation (Lipton *et al*., 1993). Presently, over 3,000 proteins have been identified as potential targets for protein S-nitrosylation (Seth and Stamler, 2011). Determination of the specific function of most protein-SNOs may be helpful for further research, and can support the notion that NO exerts its major biological activity through S-nitrosylation. Notably, S-nitrosylation mediates the protective or toxic effects of NO depending on the action of the target protein. NO produced from NOS efficiently S-nitrosylates neighboring proteins to produce protein-SNO. Along with the proteins located near NOS, NO can react to form low-molecular weight SNOs with cysteine and glutathione (GSH). These low-molecular-weight SNOs, such as S-nitrosocysteine (CysNO) and S-nitrosoglutathione (GSNO), can then function as NO donors under physiological conditions depending on their redox potential (Hess *et al*., 2005). Until very recently, transnitrosylation, which is the transfer of an NO group from one thiol to another, was believed to take place only between low-molecular-weight SNOs and protein thiols. Some leading groups in this field, however, discovered that transnitrosylation between cell proteins could be a very important enzymatic process of S-nitrosylation (Pawloski *et al*., 2001; Mitchell and Marletta, 2005; Benhar *et al*., 2008; Kornberg *et al*., 2010; Nakamura *et al*., 2010; Wu

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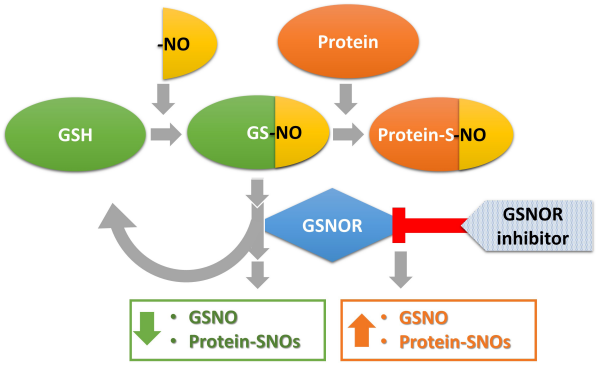

Fig. 1. GSNOR controls protein-SNOs by metabolizing GSNO. Metabolizing GSNO is one of the main functions of GSNOR. GSNOR inhibitors increase available GSNO and total protein-SNOs.

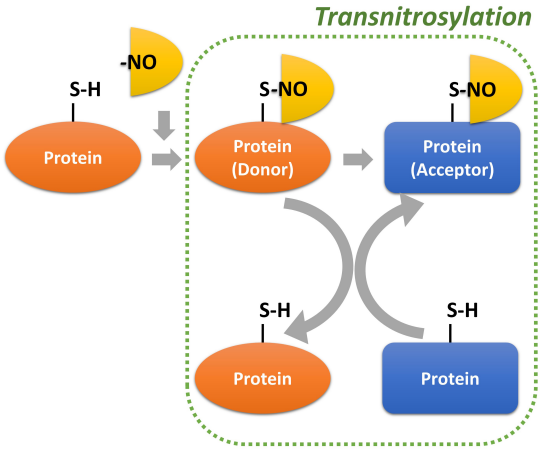

Fig. 2. Proposed mechanism of transnitrosylation. Schema illustrates transnitrosylation of an 'acceptor' protein (blue) by another S-nitrosylated 'donor' protein (orange).

et al., 2010; Qu *et al*., 2011). GSNO, a major NO donor in the physiological system, has its own special metabolic process (Fig. 1). GSNO serves as a selective substrate for S-nitrosoglutathione reductase (GSNOR), through which it is fully reduced to glutathione disulfide (GSSG) and ammonia (NH_3). At the cellular level, GSNO is in equilibrium with multiple protein-SNOs. GSNOR directly metabolizes GSNO and indirectly controls the amount of protein-SNOs and their related signals (Beigi *et al*., 2012). Although some other enzymes have been shown to be capable of catalyzing SNO degradation *in vitro* (Trujillo *et al*., 1998; Carver *et al*., 2007), they have not yet been shown to modulate endogenous SNO levels in cells. In contrast, GSNOR has been reported to play an important role in maintaining the balance between GSNO and protein-SNO in physiological conditions (Liu *et al*., 2004; Que *et al*., 2005). In this review, we will discuss the pathophysiology of basal SNO changes caused by the decrease or deficiency of GSNOR and examine the potential of GSNOR as a drug target.

PROTEIN S-NITROSYLATION AND ITS DETECTION

S-nitrosylation is a non-enzymatic reaction that occurs within the range of physiological pH. It can control protein conformations, protein–protein interactions, and other post-translational modifications such as phosphorylation, acetylation, ubiquitination, and disulfide linkage (Hess and Stamler, 2012). Although S-nitrosylation is a generally abundant chemical reaction, initial S-nitrosylation occurs only at preferable cysteines that are proximal to NOS. A major group of S-nitrosylated proteins in this class includes NOS and NOS-interacting proteins. Recent findings on the effects of protein S-nitrosylation reveal a great impact on the related biological and pathophysiological research fields (Stamler *et al*., 1992; Foster *et al*., 2009; Broniowska and Hogg, 2012). However, detecting and quantifying protein-SNO and GSNO in experimental systems are not easy. The biotin switch technique (Jaffrey and Snyder, 2001), in which S-nitrosylated cysteine is reduced and biotinylated, offers a clear and powerful method for the qualitative detection of S-nitrosylated proteins. The biotin switch technique and all other protein-SNO measurement techniques have difficulty in identifying each S-nitrosylated thiol clearly (Giustarini *et al*., 2003). Novel techniques that are precisely quantitative are currently available (Chen *et al*., 2013; Devarie-Baez *et al*., 2013), such as tandem mass spectrometry (MS/MS) of S-nitrosylated protein thiols (Murray *et al*., 2012; Ulrich *et al*., 2013).

PROTEIN-PROTEIN TRANSNITROSYLATION

As mentioned earlier, the nitrosyl group derived from nitrosylated proteins can then be moved to a remote location via transnitrosylation. Thus, transnitrosylation is also a denitrosylation process of the donor protein, overturning the first SNO-mediated regulation (Fig. 2). To date, fewer than 10 nitrosyl donors have been identified (Hess and Stamler, 2012). The recently discovered protein-protein transnitrosylation reveals that the NO moiety is transferred from protein-SNO to the free thiol of another protein, which regulates the NO regulatory mechanism of specific signaling pathways (Nakamura *et al*., 2010; Choi *et al*., 2014). Protein-to-protein transnitrosylation generally occurs when two proteins directly interact and have the appropriate redox potentials to allow electron transfer. In other words, protein binding promotes NO transfer between the two proteins. It is currently believed that physical association of two proteins could promote conformational change, thereby affecting the environment of crucial cysteine residues to assist thiolate anion formation, which is more responsive to S-nitrosylation, followed by further oxidation by ROS. If a protein-SNO interacts with a partner protein having free thiols, the difference in redox potential of the two cysteine residues is a primary determinant of NO transfer. Proteins with higher redox potential tend to be reduced by receiving electrons, which means that they are being denitrosylated. Thus, when a protein with a lower redox potential having a free thiol enclosed by suitable amino acid motifs for S-nitrosylation interacts with a previously nitrosylated protein, transnitrosylation would follow. Considering the above, only a certain subset of proteins is S-nitrosylated to selectively activate or inhibit specific signaling pathways.

DUAL ROLE OF PROTEIN S-NITROSYLATION DEPENDING ON NO LEVEL

NO often mediates cytoprotection at the physiological level depending on the cellular compartments involved. The most representative example of this protective effect can be found in the relationship between NO/SNO signaling pathway and NMDAR. NO reacts with NMDAR to downregulate its excessive activity (Takahashi *et al*., 2007). S-nitrosylation of NMDAR under excitotoxic conditions, wherein neurotoxic cells are damaged by excessive activation of glutamate receptors, can provide a neuroprotective effect (Lipton *et al*., 1993). Notably, it has been found that Cys399 on the NR2A subunit promotes almost 90% of NO effect under ambient conditions, although NO also causes S-nitrosylation of the other four cysteine residues on extracellular domains of NR1 and NR2 subunits of NMDAR (Choi *et al*., 2000). Based on the crystal structure models and electrophysiological experiments, it has been found that S-nitrosylation of Cys399 can induce conformational changes of the receptor, thereby enhancing Zn^{2+} and glutamate binding to the receptor, resulting in receptor desensitization and subsequent ion channel closing (Lipton *et al*., 2002). In contrast, excessive levels of NO are believed to activate toxic signaling pathways depending on the source and location of NO production. Occasionally, for example, NO reacts rapidly with superoxide anions produced from mitochondrial and/or non-mitochondrial sources to form the highly toxic peroxynitrite ($ONOO^-$) (Lipton *et al*., 1993; Brennan *et al*., 2009). Moreover, it has been shown that excessive oxidative/nitrosative stress, which could be pathological, causes cell death through S-nitrosylation of multiple targets such as parkin, dynamin related protein 1 (Drp1), protein disulfide isomerase (PDI), X-linked inhibitor of apoptosis (XIAP), matrix metalloproteinase-9 (MMP-9), cyclooxygenase-2 (COX-2), N-ethylmaleimide sensitive factor (NSF), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Lipton *et al*., 1993; Gu *et al*., 2002; Hara *et al*., 2005; Huang *et al*., 2005; Tian *et al*., 2008). Recent reports suggest that excessive NO-related species may play a critical role during the process of protein misfolding and lead to neuronal damage through S-nitrosylation of PDI and parkin. In this case, protein S-nitrosylation decreases the isomerase and chaperone activities of PDI (Uehara *et al*., 2006), resulting in the accumulation of misfolded proteins and subsequent cytotoxicity in neurodegenerative disease models. It has also been shown that S-nitrosylation of parkin affects its E3 ligase activity, possibly affecting Lewy body formation in Parkinson's disease (Chung *et al*., 2004; Yao *et al*., 2004).

REGULATION OF BASAL NO/SNO BY GSNOR

In humans, GSNOR is encoded by alcohol dehydrogenase 5 (ADH5) gene (Smith, 1986). Unlike other ADHs, whose expression is mostly tissue-specific, GSNOR is abundantly expressed in all tissues (Hur and Edenberg, 1992; Höög and Östberg, 2011). Almost all studies on the activity and regulation of GSNOR are strongly associated with protein S-nitrosylation. GSNOR is a negative regulator of GSNO in smooth muscles (Que *et al*., 2009), and the abnormal expression of ADH5 is strongly related to disease development (Jelski and Szmitkowski, 2008; Jelski *et al*., 2009; Laniewska-Dunaj *et al*., 2013). The effects of GSNOR-deficient experimental systems are listed in Table 1. Deletion of ADH5 gene increases not only the amount of GSNO, but also total protein S-nitrosylation *in vivo* (Liu *et al*., 2001). Protein S-nitrosylation is very important for researchers and clinicians because hypo- or hyper-S-nitrosylation of various protein sets across almost all tissue types can have a large impact on specific diseases (Foster *et al*., 2009), such as type 2 diabetes (Carvalho-Filho *et al*., 2005), sickle cell anemia (Bonaventura *et al*., 2002), ventricular arrhythmia in individuals with Duchenne muscular dystrophy (Fauconnier *et al*., 2010), cell death and survival pathways (Iyer *et al*., 2014), post-infarct cardio-protection (Methner *et al*., 2014), and pregnancy/parturition (Ulrich *et al*., 2013). GSNOR itself is another cysteine-rich protein that is S-nitrosylated by GSNO. Consequently, a feedback loop affecting GSNOR expression and activity can be initiated (Brown-Steinke

Table 1. Effects of GSNOR-deficient experimental systems. Positive or negative effects caused by GSNOR deficiency were listed by organs

Organs	Phenotypes	Effects	References
Brain	Neuronal differentiation	Increased	Wu *et al*., 2014
	Neuroprotection against PD toxins	Increased	Clements *et al*., 2006
	Visual pattern memory	Decreased	Hou *et al*., 2011
Thymus	Mortality upon endotoxic shock or bacterial challenge	Increased	Liu *et al*., 2004
	B and T lymphocyte development	Decreased	Yang *et al*., 2010
Lungs	Bronchodilation	Increased	Que *et al*., 2005
	Protection against experimental asthma	Increased	Que *et al*., 2005
Heart	Retention of cardiac function after ischemia	Increased	Lima *et al*., 2009
	Cardiomyocyte proliferation	Increased	Hatzistergos *et al*., 2015
Liver	Hepatic progenitor cells proliferation during development	Increased	Cox *et al*., 2014
	Hepatoprotection against acetaminophen intoxication	Increased	Cox *et al*., 2014
	Incidence of spontaneous hepatocellular carcinoma (HCC)	Increased	Wei *et al*., 2010
Skeletal muscle	Strength and fatigue resistance	Increased	Moon *et al*., 2017
	Myofiber size and muscle efficiency	Decreased	Montagna *et al*., 2014
Blood vessels	Vasculogenesis	Decreased	Gomes *et al*., 2013
	Peripheral vascular tone and β-adrenergic response	Decreased	Beigi *et al*., 2012

et al., 2010; Guerra *et al*., 2016). Taken together, dysregulation of GSNOR is associated with several human diseases. By using ADH5$^{-/-}$ animal models, critical data regarding GSNOR function were obtained. NO-mediated pathway as well as protein-SNO levels are severely affected when GSNOR activity is changed.

GSNOR AS A THERAPEUTIC TARGET

When GSNOR activity was regulated, not only the protein-SNO level, but also the NO-mediated pathway varied markedly. GSNOR inhibition increased intracellular GSNO availability and promoted NO-mediated signal transduction pathways. Drugs that can inhibit the function of GSNOR have been studied (Sanghani *et al*., 2009; Green *et al*., 2012; Sun *et al*., 2012; Jiang *et al*., 2016). Among them, N6022 and N91115 have been tested in both mild asthma and cystic fibrosis, and have been proved to be potentially safe and effective GSNOR inhibitors. In patients with cystic fibrosis, endogenous GSNO levels were low (Grasemann *et al*., 1999) and GSNOR inhibition was relatively more effective than direct administration of GSNO (Zaman *et al*., 2001; Snyder *et al*., 2002). Since there is no FDA-approved GSNOR inhibitor currently available, attention has been focused on the clinical use of existing drugs that show effects on modulating S-nitrosothiols (RSNOs). The β1-adrenergic receptor blocker, nebivolol, used in the treatment of hypertension has been shown to increase total RSNO levels in animal and cell models (Jiang *et al*., 2016). However, when GSNOR inhibitors are considered as therapeutic agents, it is necessary to consider the level of intracellular NO that can be controlled by enzymes other than GSNOR. Since NO is crucial to the normal functioning of most cell types, there are several complementary mechanisms that regulate NO and RSNO, such as thioredoxin (Trx) (Sengupta and Holmgren, 2013) and carbonyl reductase systems (Bateman *et al*., 2008). Nevertheless, the direct administration of endogenous NO donors and some exogenous NO donors is not clinically valuable because of rapid degradation and serious side effects such as systemic nitrate accumulation (Al-Sa'doni and Ferro, 2005). Therefore, the therapeutic inhibition of GSNOR for the treatment of patients should be carefully considered in view of potential side effects.

CONCLUSION AND FUTURE PERSPECTIVES

Recently, S-nitrosylation has been considered an essential post-translational modification of reactive cysteines. Many proteins have been discovered to be S-nitrosylated, which results in a change in their activity and function. As denitrosylation has been shown to be catalyzed by specific enzymes, Trx and GSNOR have been discovered as enzymes that remove NO from nitrosylated proteins. The discovery of these novel denitrosylation systems have opened new arenas in redox biology and have promoted application studies on related biological and pharmacological signaling pathways. To date, several studies have shown the results of GSNOR-mediated cellular processes or the phenotypes caused by GSNOR gene deletion (Hess and Stamler, 2012). Despite the large amount of information on the involvement of GSNOR in physiological processes, the mechanism by which GSNOR mediates selective denitrosylation is still unclear. By elucidating the mechanism by which GSNOR can act as a selective enzyme, we can expect that the NO/SNO pathway would become a more important target of disease treatment. Given these aspects, it is important to determine the value to be investigated to identify potential post-translational modifications that can modulate GSNOR localization or interaction with other proteins, and to provide a better understanding of future S-nitrosylation dynamics and signaling pathways. Obtaining a better understanding of the precisely regulated denitrosylation pathways and their clinical significance will help not only to discover new targets for drug action, but also to develop new therapeutic agents.

CONFLICT OF INTEREST

There is no conflict of interest.

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