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Forum Review

Chemical Considerations and Biological Selectivity of Protein Nitrosation: Implications for NO-Mediated Signal Transduction

BENJAMIN GUIKEMA, QI LU, and DAVID JOURD'HEUIL

ABSTRACT

Nitric oxide (NO) is a diatomic free radical that plays an important role in the homeostatic regulation of the central nervous, immune, and cardiovascular systems. In addition to its interaction with guanylate cyclase, which results in the production of the second messenger cyclic GMP, there is now a large body of literature indicating that many of the effects associated with the production of NO are due to the nitrosation of cysteine residues in proteins. In this review, we outline the primary chemical pathways that may account for protein nitrosation in cells and tissues. The functional implications of protein nitrosation are discussed by using the $p21^{ras}$ subfamily of small monomeric GTPases and the cysteine-containing aspartate-specific proteases (caspases) as prototypical examples. Overall, in addition to the well characterized NO/O_2 reaction, there may exist multiple pathways accounting for protein nitrosation in cells. These include acid- and free radical-mediated mechanisms. Although protein nitrosation may not be limited to cysteine residues, there is now ample evidence that nitrosation reactions, in a fashion similar to oxidative modifications, may modulate the structure, activity, association, and localization of a specific subset of proteins in cells and tissues. *Antioxid. Redox Signal.* 7, 593–606.

INTRODUCTION

THE CONCEPT THAT NITRIC OXIDE (nitrogen monoxide, NO) regulates biological processes by directly or indirectly modifying amino acid residues in peptides and proteins has gained much attention in recent years. Considerable focus is now placed on the covalent attachment of NO moieties to the sulfhydryl group of cysteine residues as an important post-translational modification to regulate protein function and signal transduction pathways (69, 79). Nitrosation is defined in chemical terms as the addition of a nitrosonium (NO^+) equivalent to a functional group such as a thiol to form a nitrosothiol (RSNO). Nitrosylation refers to the addition of a molecule of NO to a group such as a metal to form nitrosyl or nitroso species. The term nitrosylation is also used as an alternative to indicate the multiplicity of mechanisms leading to the formation of nitroso species, including RSNOs. Overall, the formation of RSNOs is documented in humans and in a number of animal models, and numerous investigations have directly or indirectly implicated RSNOs in intracellular signal transduction pathways in cell systems (6, 19, 23, 26, 53, 105). Clearly, without a detailed understanding of the mechanisms by which the NO moiety may be attached and removed from cysteine residues, it will be difficult to evaluate how RSNOs regulate cell function. The aim of this review is to give an overview of the chemical pathways leading to RSNO formation and to focus on recent developments, which evaluate the importance of the cellular environment in modulating RSNO formation and decomposition. We will also describe the possible relevance of RSNO formation in regulating key signal transduction proteins, including Ras and caspases.

MECHANISMS OF RSNO FORMATION

The pathways leading to RSNO formation have been examined in some detail *in vitro.* In many cases, cysteine and the tripeptide glutathione (GSH) have been used as model compounds to study the effect of NO itself or reactive nitro-

Center for Cardiovascular Sciences, Albany Medical College, Albany, NY.

gen species (RNS) derived from the secondary reaction of NO with, for example, molecular oxygen (O_2) or superoxide ($O_2^{\cdot -}$). Whether these pathways are relevant in cells and tissues is, in fact, largely unknown, and the discussion below provides clues as to the biological context in which certain of these reactions may prevail.

Acidic nitrosation

The most facile pathway leading to RSNO formation is through the reaction of thiols with nitrous acid (HNO_2) formed from acidified nitrite [NO_2^-; (114)]. Dinitrogen trioxide (N_2O_3) is classically viewed as the nitrosating agent generated:

$$NO_2^- + H^+ \leftrightarrow HNO_2 \quad (1)$$

$$2HNO_2 \leftrightarrow N_2O_3 + H_2O \quad (2)$$

$$N_2O_3 + RSH \rightarrow RSNO + NO_2^- + H^+ \quad (3)$$

As the pK_a for HNO_2 is 3.4, most of the acid is dissociated to NO_2^- at neutral pH. This suggests that the primary relevance of this chemistry is in tissues that maintain a low pH. This includes the acidic stomach, although RSNO formation within this context has been much less studied than that of the nitrosation of amines and the formation of carcinogenic *N*-nitroso compounds (84). In addition, inflammatory conditions that may favor acidification, such as urinary and airway inflammation, might be associated with increased nitrosation (99). Clearly, the acidification of the phagosomal lumen of activated leukocytes such as neutrophils and macrophages (34) might promote nitrosation reactions, and in this way NO_2^- acidification may participate in bacterial killing (62).

Direct reaction of NO with thiols

Under strict anaerobic conditions, NO reacts directly with thiols to yield nitrous oxide (N_2O) and thiol oxidation products (45, 94). In this case, the reaction of NO with GSH (or its conjugated thiolate, GS^-) yields glutathione disulfide (GSSG) through the proposed intermediate formation of hyponitrous acid (HONNOH):

$$GSH + NO \leftrightarrow GSNOH \quad (4)$$

$$2GSNOH \rightarrow GSSG + HONNOH \quad (5)$$

$$HONNOH \rightarrow N_2O + H_2O \quad (6)$$

$$GSNOH + NO \rightarrow GSOH \quad (7)$$

$$GSOH + GSH \rightarrow GSSG + H_2O \quad (8)$$

At 5 m*M* GSH, the overall pseudo-first-order rate of the reaction is 4.8×10^{-4} s^{-1}, suggesting that the anaerobic oxidation of thiols by NO might be of limited physiological importance (45). Aravindakumar and co-workers provided evidence to suggest otherwise at least at high output of NO (1). The reaction of NO with thiols does not appear to produce RSNOs (45). However, electron acceptors such as NAD^+ may facilitate RSNO formation through reaction with the nitrogen-centered free radical intermediate (RSNOH) produced by the initial reaction of the thiol with NO (32):

$$RSNOH + NAD^+ \rightarrow RSNO + NADH \quad (9)$$

Experimental evidence for Reaction 9 is based on the measurement of the rate of NO disappearance in solution in the presence of deoxygenated GSH and NAD^+, which evidently warrants further characterization and direct RSNO determination in order to verify the relevance of this process (32).

Metal-catalyzed nitrosation

The chemistry derived from the interaction between NO and metals is complex and provides the chemical foundation for many of the functional effects of NO. Metals may favor either the formation or degradation of RSNOs. Physiologically relevant NO carriers such as dinitrosyl iron complexes have been regarded as a source of NO^+ in cells and tissues (5), and the proposal that hemoglobin produces RSNOs has been much debated in recent years (31). Thus, iron(III) complexes such as porphyrins and heme proteins activate NO to yield a RNS, which nitrosates thiols as well as amines (112). Iron(II) may then recapture a molecule of NO to form a nitrosyl complex:

$$Iron(III) + NO \rightarrow Iron(II)NO^+ \quad (10)$$

$$Iron(II)NO^+ + RS^- \rightarrow Iron(II) + RSNO \quad (11)$$

$$Iron(II) + NO \rightarrow Iron(II)NO \quad (12)$$

Protein-associated copper may also serve as an electron acceptor to oxidize NO to nitrosonium (NO^+), which in turn nitrosates thiols. This has been demonstrated for albumin (107) as well as GSH in the presence of ceruloplasmin, a multicopper-plasma protein (47). Conversely, Dicks and Williams have established that protein-associated copper may also mediate RSNO decomposition (12). They determined that this occurs via the reduction of Cu^{2+} to Cu^+ by a reducing agent such as GSH, with the subsequent decomposition of the RSNO (12, 13). Overall, these observations suggest that certain metal-containing proteins may regulate the steady-state concentration of intracellular RSNOs. However, to date, there is little indication to support this contention in cells and tissues.

Transnitrosation

Transnitrosation is the reversible transfer of a nitroso group between two thiolates in the absence of the apparent release of NO:

$$RSNO + R'S^- \leftrightarrow RS^- + R'SNO \quad (13)$$

Transnitrosation reactions are reasonably well characterized for a number of low-molecular-weight (LMW) RSNOs, such as *S*-nitrosocysteine (CysNO) and *S*-nitrosoglutathione (GSNO) (44, 77, 82, 124). The rate constants for these reactions are classically slow (10^1–10^3 $M^{-1}s^{-1}$), which in many cases might be compensated by the relative abundance of thi-

ols. It is important to mention that transnitrosation is not the only relevant chemistry derived from the reaction of thiols with RSNOs because this reaction is also associated with nitroxyl anion (NO^-) and disulfide formation (103, 119). In the bloodstream, we have shown that albumin serves as a sink for physiological and pharmacological concentrations of LMW RSNOs through transnitrosation reactions (54). Alternatively, manipulations that increase circulating LMW thiols may result in the mobilization of NO pools by shifting equilibriums from *S*-nitrosoalbumin toward the formation of LMW RSNOs (102). In the intracellular environment, GSH is the most abundant LMW thiol with concentrations estimated between 1 and 10 m*M*. However, protein thiols are still in far excess, and RSNO levels may be shifted toward protein RSNOs, which may explain the absence of any measurable levels of GSNO in cells. It is important to remember that transnitrosation proceeds through the nucleophilic attack of a thiolate on the nitrogen of the RSNO and therefore depends on the pK_a of the thiol group. As in many cases the protein environment favors thiols with low pK_a, this may further enhance the overall transfer of NO^+ equivalents from LMW thiols to proteins. Of note is the observation that cell-surface protein disulfide isomerases catalyze transnitrosation reactions, suggesting that extracellular NO and RSNOs may gain access to the intracellular environment through these reactions (98, 120).

RSNO and NO autoxidation

RNS derived from the reaction of NO with O_2 nitrosate thiols. The reaction is second order with respect to NO and first order in O_2, consistent with the formation of N_2O_3 as the nitrosating agent (28, 57, 116).

$$2NO + O_2 \rightarrow 2NO_2 \quad (14)$$

$$2NO + 2NO_2 \rightarrow 2N_2O_3 \quad (15)$$

$$N_2O_3 + RSH \rightarrow RSNO + NO_2^- + H^+ \quad (16)$$

The second order of Reaction 14 with regard to NO indicates that the rate of RSNO formation is controlled by the local concentration of NO and that N_2O_3 would form quantitatively only when the concentration of NO rises to the micromolar range (3). *In vivo* conditions associated with the upregulation of the inducible form of NO synthase (iNOS) are accompanied by the production of such levels. The oxygen dependence of this process also suggests that O_2 concentrations, which are in the range of 1–50 μ*M* in most tissues (50), will greatly limit the efficacy of this reaction. Obviously, the pulmonary alveoli and associated capillaries, where the P_{O_2} is close to that of alveolar and ambient air, may provide a promiscuous environment for NO/O_2-mediated reactions in as much as resident macrophages might be already primed to produce large amounts of NO (33). Therapeutic use of inhaled NO and inflammatory settings such as asthma where NO production might be further enhanced would also lead to conditions that may facilitate this pathway (24, 46).

Lancaster and co-workers demonstrated that hydrophobic environments such as membrane bilayers represent important sites for nitrosation reactions because the NO/O_2 reaction is accelerated ~300-fold in hydrophobic phases (76). The reaction is second order relative to NO, and the final oxidation product is NO_2^-, similar to NO autoxidation in aqueous solution. Thus, the same RNS [nitrogen dioxide (NO_2) and N_2O_3] generated during NO autoxidation in solution might be produced within the lipidic environment, albeit at a greater rate. Thus, membrane proteins would be primary targets for nitrosation, but evidence for an increased susceptibility of membrane protein thiols versus thiols in the surrounding aqueous medium is still lacking (125, 126).

We recently reinvestigated RSNO formation in oxygenated solution in the presence of excess thiols relative to NO, a condition that approximates to some extent conditions in the intracellular environment (56). We showed that NO autoxidation resulted in the formation of GSSG in amounts that exceeded those of GSNO. This could be rationalized if a large fraction of NO_2 generated through Reaction 14 were consumed by reacting with excess GSH to yield the glutathionyl radical [GS•(21, 113)]. It also follows that a fraction of GSNO might be formed through direct combination of GS• with NO and that peroxynitrite might be produced through the chemistry derived from the reaction of the thiyl radical with GSH and O_2 (Fig. 1). An important point from this study is that oxidation is always associated with the NO/O_2 reaction in cells and tissues in amounts that in fact exceed that of nitrosation (56). Our data would support the concept that protein thiol oxidation (and glutathionylation) through RNS derived from the NO/O_2 reaction is as relevant as nitrosation with regard to the modulation of protein and cell function.

Although both NO_2 and N_2O_3 have a high affinity for thiols, other relevant substrates will effectively compete to limit protein nitrosation (117). Physiological concentrations of phosphate, chloride, and bicarbonate all inhibit N_2O_3-mediated nitrosation reactions (8, 71) although this may vary with the reactants [*i.e.,* amine versus thiols (52)]. *In vivo,* intracel-

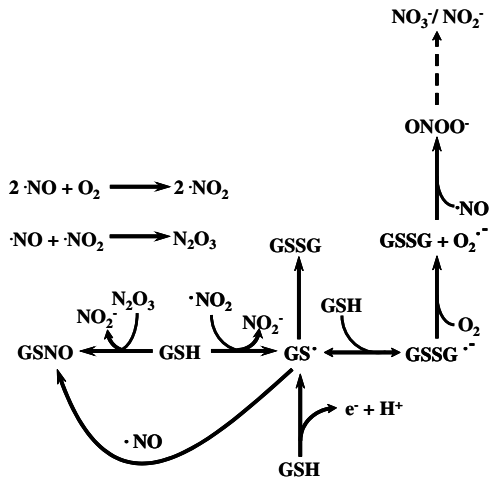

FIG. 1. Reaction of NO with GSH in oxygenated solution. The reaction of NO with excess GSH yields GSNO and GSSG. GSSG is formed from the one-electron oxidation by nitrogen dioxide (NO_2) of GSH to yield the thiyl radical GS•. NO may be consumed by reaction with superoxide ($O_2^{\bullet -}$), formed from the reduction of molecular oxygen (O_2) by the glutathione disulfide anion radical ($GSSG^{\bullet -}$). See text for details.

lular ascorbate concentrations equal or exceed those of GSH, which provide additional competitive pathways (63, 73). In membranes, unsaturated fatty acyl chains as well as tocopherol may also greatly affect the chemistry associated with NO autoxidation by directly scavenging RNS (91).

Reaction of peroxynitrite with thiols

One of the most significant reactions of NO is its combination with $O_2^{\bullet-}$ to yield peroxynitrite [$ONOO^-$/ONOOH (4)]. The reaction of peroxynitrite with thiols leads primarily to the accumulation of oxidized products (95, 96). RSNOs are also formed albeit at low yields (*i.e.,* 1–2% relative to peroxynitrite), possibly through the intermediate formation of a thionitrate [$RSNO_2$ (2)]. Alternatively, RSNO might be formed via direct nucleophilic nitrosation with elimination of hydrogen peroxide (111):

$$ONOOH + RS^- \rightarrow RSNO_2 + OH^- \quad (17)$$

$$RSNO_2 + RSH \rightarrow RSOH + RSNO \quad (18)$$

$$RS^- + ONOOH \rightarrow RSNO + HOO^- \quad (19)$$

In either case, the presence of excess bicarbonate is inhibitory, suggesting that peroxynitrite-mediated nitrosation may not proceed in the CO_2-rich extracellular environment. LMW compounds, such as uric acid, and glucose enhance thiol nitrosation via the intermediate formation of alkyl nitrites (86, 104). Ng and co-workers found that perfusion of superoxide dismutase (SOD) in cats breathing NO inhibited albumin nitrosation after ischemia–reperfusion, suggesting a role for $O_2^{\bullet-}$ and/or peroxynitrite in modulating circulating RSNOs *in vivo* (90). Thus, although the yields of nitrosation by peroxynitrite may be low, the relative stability of certain RSNOs may allow for their accumulation even through peroxynitrite-mediated reactions.

One important aspect of peroxynitrite chemistry *in vivo* is that it is presumably the result of the simultaneous generation of $O_2^{\bullet-}$ and NO. This is most important during inflammation, where the production of both radicals is increased and peroxynitrite may thus be formed in large quantities. However, it is possible to argue that the complexity and heterogeneity of inflammatory loci is such that conditions for the formation of peroxynitrite may never be optimal, which might by itself limit the impact of peroxynitrite. Thus, peroxynitrite might always be formed in the presence of excess NO or $O_2^{\bullet-}$. Macrophages, neutrophils, and other inflammatory cells produce $O_2^{\bullet-}$ and NO in amounts that are clearly cell type-dependent. For example, neutrophils when fully activated produce 100–1,000 times more $O_2^{\bullet-}$ than NO. In addition, it takes several hours to up-regulate iNOS expression, whereas the phagocytic NADPH oxidase can be activated within seconds to produce $O_2^{\bullet-}$. The different temporal and spatial regulation of NO and $O_2^{\bullet-}$ production is of particular interest because recent studies indicate that the cogeneration of NO and $O_2^{\bullet-}$ modulates the chemistry associated with peroxynitrite production (29, 41, 55, 83, 93, 101). For example, peroxynitrite-mediated oxidation reactions are inhibited in the presence of excess NO or $O_2^{\bullet-}$, because both NO and $O_2^{\bullet-}$ scavenge the free radical intermediates formed from the self-decomposition of peroxynitrite (55). Excess $O_2^{\bullet-}$ also inhibits the nitrosation of GSH mediated by the NO/O_2 chemistry by directly competing with O_2 for NO (118). As the yields of nitrosation through peroxynitrite are much lower than through NO/O_2, there is a consequential decrease in RSNO yields. In the presence of excess NO, scavenging of NO_2 formed from the self-decomposition of peroxynitrite or from the reaction of peroxynitrite with CO_2 yields N_2O_3, thereby redirecting the oxidative chemistry associated with peroxynitrite to the nitrosation of thiols by N_2O_3 (118). However, as noted earlier, the NO/O_2 reaction in the presence of excess thiol is also accompanied by substantial amounts of oxidation such that the dichotomy between oxidative (peroxynitrite-mediated) stress and nitrosative (NO/O_2-mediated) stress may not be as evident as previously thought (Fig. 2).

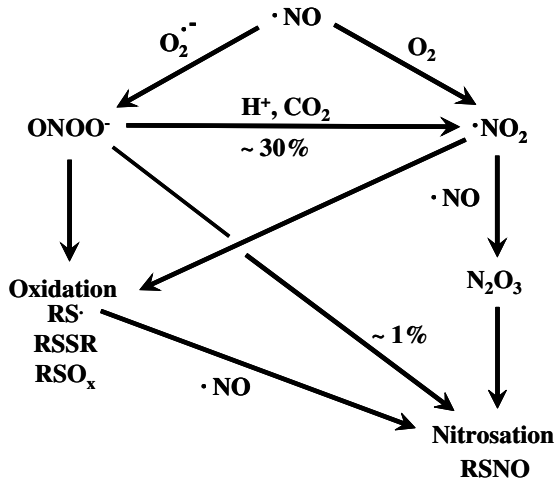

FIG. 2. Nitrosation versus oxidation of thiols. The reaction of NO with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$) may redirect the nitrosative chemistry associated with NO and O_2 to a more oxidative one. As the yields of nitrosation through peroxynitrite (~1%) are much lower than through NO/O_2, there is a consequential decrease in RSNO yields in the presence of excess $O_2^{\bullet-}$. There is also evidence to suggest that NO_2 derived from either NO/O_2 or $NO/O_2^{\bullet-}$ is an important contributor to the oxidative chemistry associated with these two pathways. See text for details.

It is possible to argue that under physiological conditions, peroxynitrite does not coexist with excess NO because oxyhemoglobin insures limited availability of NO in vascularized tissues due to rapid reaction to form NO_3^- (Fig. 3). In cells, $O_2^{\bullet-}$ production is buffered by superoxide dismutase (SOD) such that peroxynitrite formation is primarily dependent on NO availability and not $O_2^{\bullet-}$ (Fig. 3). In addition, the inability of $O_2^{\bullet-}$ to diffuse through membrane bilayers limits the site of peroxynitrite formation and reactivity to finite locations, although peroxynitrite itself and some of its decomposition products might diffuse through membrane bilayers to some extent (87). However, during pathologic conditions such as acute and chronic inflammation, which are characterized by unchecked high NO synthase (NOS) activity and mul-

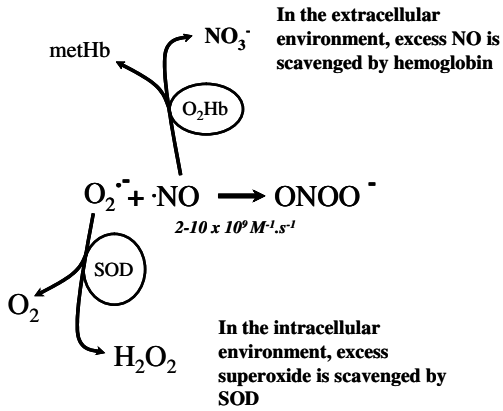

FIG. 3. The formation of peroxynitrite ($ONOO^-$) in the presence of excess NO or $O_2^{\cdot-}$ may be limited by (SOD) and oxyhemoglobin. See text for details.

tiple cellular sources of $O_2^{\cdot-}$, modulation of peroxynitrite-mediated reactions by NO or $O_2^{\cdot-}$ may become important. Thus, any disruption of steady-state levels of NO and/or $O_2^{\cdot-}$ through either increased production or inactivation of scavenging systems such as superoxide dismutase will increase the prevalence of the secondary reactions described earlier and modulate peroxynitrite reactivity.

Free radical nitrosation

The existence of multiple competitive pathways for the utilization and consumption of NO makes it difficult to rationalize the presence of significant amounts of RSNOs *in vivo.* One-electron oxidation of thiols to form thiyl radicals ($RS^{\cdot}$) is a common accompaniment of oxygenated environments, and the combination of the free radical NO with another free radical is thermodynamically and kinetically favorable. It follows that the reaction of a thiyl radical with NO might provide a competitive advantage for the formation of RSNOs *in vivo*:

$$RSH \rightarrow RS^{\cdot} + H^+ + e^- \quad (20)$$

$$RS^{\cdot} + NO \rightarrow RSNO \quad (21)$$

In the case of GSH, we have shown that GSNO formation in oxygenated solution of NO may occur upon oxidation of GSH by NO_2 to form the glutathionyl radical, which in turn directly reacts with NO to form GSNO (Fig. 1; 56). NO may also directly combine with oxygenated radical products such as $RSO^{\cdot}$:

$$RSO^{\cdot} + NO \rightarrow RSONO \quad (22)$$

$$RSONO + RSH \rightarrow RSNO + RSOH \quad (23)$$

There is, however, very limited experimental evidence to support the concept that the one-electron oxidation of thiols may lead to increased RSNO levels during conditions of increased oxidative stress. For thiol sites that may be available for reaction with GSH, it is possible that RSNO formation may be limited by the competitive reaction of the thiyl radical with excess GSH to form disulfide anion free radical (Fig. 1). This intermediate reduces O_2 to $O_2^{\cdot-}$, which may provide a sink for NO by forming peroxynitrite, a poor nitrosating agent (compared with RNS derived from the NO/O_2 reaction).

$$RS^{\cdot} + GS^- \rightarrow RSSG^{\cdot-} \quad (24)$$

$$RSSG^{\cdot-} + O_2 \rightarrow RSSG + O_2^{\cdot-} \quad (25)$$

$$O_2^{\cdot-} + NO \rightarrow ONOO^- \quad (26)$$

Thus, the free radical pathway to yield RSNOs may be significant only if the thiol target is not accessible to GSH or if GSH is depleted or present at a low concentration. During conditions of oxidative stress, depletion of antioxidants such as GSH and ascorbate may favor the nitrosation of proteins by allowing the initial oxidation of thiol groups in proteins to thiyl radical, which may further combine to yield RSNOs.

INTRACELLULAR NITROSATION

The question that arises from the above discussion is whether the chemical pathways derived from *in vitro* experiments apply to the intracellular environment. For example, Kim and co-workers found that preloading macrophages with iron increased RSNO formation, an effect associated with the inhibition of the proapoptotic enzyme caspase-3 (61). This interesting study is, to our knowledge, the only work that has examined the impact of intracellular iron on nitrosation in cells. Similarly, although RNS derived from NO autoxidation are potent nitrosating agents, there is, however, very limited information as to the relevance of this pathway in cells and tissues (56). In fact, the nature of the nitrosating species derived from the NO/O_2 reaction has been proposed to differ between cells and the aqueous environment (16), suggesting discrete subcellular localization of nitrosation patterns. Differential susceptibility of protein nitrosation in cells is also highlighted in a recent study by Foster and Stamler, who identified a subset of mitochondrial proteins that were nitrosated under anaerobic conditions, whereas a larger and different pool were nitrosated in the aerobic environment (22).

What we do know is that, in model systems using cells in suspension or adherent cells, nitroso species are consistently associated with the high-molecular-weight (HMW) fraction (>3,000 Da), and in most studies >80% of the nitrosated fraction is sensitive to mercury, indicating that the large majority of these compounds are indeed S-nitrosothiols (17, 42, 56, 127). In this regard, cell culture experiments differ from tissue determination experiments, where a much larger fraction of the nitroso species is mercury-resistant (6). This would suggest that other derivatives, such as *N*-nitrosamines (RNNOs), might be abundant *in vivo,* but the reasons for the apparent discrepancy between the cell and *in vivo* systems are unknown. A possible explanation might be related to differences in targets (for example, the extracellular matrix from adherent cells *in vitro* is usually limited), as well as key differences in the chemical pathways leading to RNNOs and RSNOs formation *in vivo* and *in vitro.*

Another general observation is that nitroso species represent only a very small fraction of total NO produced or added to the system. Hogg and co-workers estimated that the fraction of RSNOs formed from stimulated macrophages represents only 0.02% of the total NO_2^- generated by these cells (127). Considering that an important fraction of NO produced under these conditions may evolve to nitrate (NO_3^-) or may be lost through diffusion in the gas phase, this would then represent an upper limit. It can be argued that the large excess of NO_2^- and NO_3^- compared with RSNOs is due to inherent limitations of studying cells *in vitro*. This is because the excess medium covering the cells is such that most of the NO added or produced might be forced to autoxidize to NO_2^- (9). However, Bryan and co-workers also found as much as 1,000-fold excess NO_2^- and NO_3^- in tissues relative to RSNOs (6), in agreement with previous work that examined the distribution of NO metabolites in the circulation (27, 70).

GSH is thought to be an important detoxifier of reactive oxygen and nitrogen species (Fig. 4). However, measurements of intracellular RSNO have failed consistently to detect significant amounts of GSNO (or any other LMW RSNOs) despite estimates of GSH concentration ranging from 0.1 to 10 m*M* (17, 42, 127). The reason for this discrepancy is generally ascribed to the fact that protein thiols are preferential targets of RNS due to their lower pK_a and to the resultant increase in reactivity toward electrophiles. Rather than a direct target, GSH may serve as a repair system to form mixed disulfide with protein thiols (Fig. 4). Work by Clancy and co-workers indicated that exposure of neutrophils to NO resulted in decreased intracellular GSH, which was ascribed originally to the formation of GSNO (11). However, the method utilized to measure GSNO did not discriminate between RSNOs and protein-glutathione mixed disulfide (92). Similarly, treatment of cells with large amounts of exogenous RSNOs is accompanied by the formation of glutathionylated and cysteylated proteins (78). Overall, these studies would point to protein *S*-thiolation as an important modification derived from NO, and it is possible that some of the functional alterations observed with NO may be due to protein-mixed disulfide rather than RSNOs.

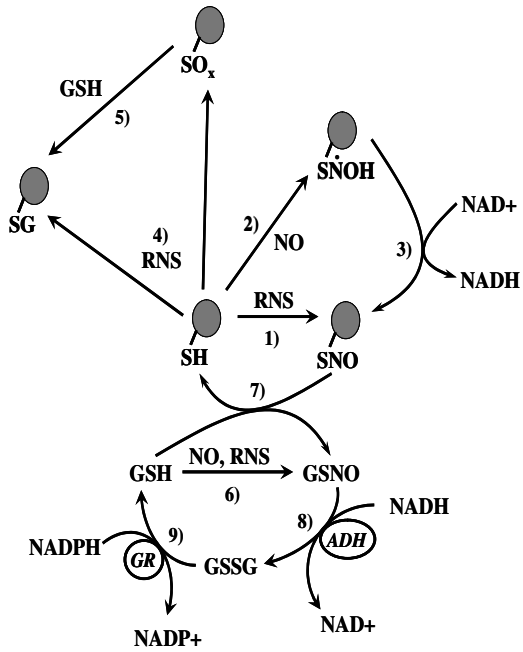

FIG. 4. Relationship between NO-mediated protein modifications and GSH availability. RNS may cause the reversible nitrosation of cysteine residues in proteins (reaction 1). Under anaerobic conditions, NO may react directly with cysteine residues in protein to form a nitrogen-centered radical (reaction 2) that can evolve to a nitrosothiol (RSNO) in the presence of an electron acceptor such as NAD^+ (reaction 3). RNS may also mediate the oxidation of cysteines and the formation of mixed disulfide (reaction 4). Depending on the accessibility of the cysteine residue on the protein, GSH may serve as a "repair" mechanism to yield mixed disulfides (reaction 5). GSH may also be nitrosated through reaction with RNS (reaction 6) or transnitrosation with nitrosated proteins (reaction 7). In this manner, GSNO may serve as a detoxifying mechanism through metabolism by alcohol dehydrogenase 3 (ADH) to yield GSSG (reaction 8). GSSG is reduced back to GSH by glutathione reductase (GR; reaction 9).

Although some have argued that GSNO might not represent an important product of intracellular nitrosation, others have suggested that there might exist specific pathways to decompose GSNO rapidly, which might explain its low intracellular levels. Recent evidence suggests that alcohol dehydrogenase 3 (ADH3) serves to catalyze the reductive breakdown of GSNO (49). This occurs with a relatively high efficiency compared with other putative substrates, such as S-hydroxymethylglutathione, and other RSNOs that are not substrates (38, 49). NADH rather than NADPH is used as a cofactor, and the major breakdown products are GSH, glutathione sulfinamide, and GSSG. A similar GSNO-metabolizing activity was also purified from *Escherichia coli*, and mouse macrophages and cells deficient in ADH3 have increased levels of both LMW and HMW RSNOs after NO exposure (74). Mice with a targeted deletion of ADH3 have increased basal RSNO levels in the circulation (75). These animals display increased mortality upon endotoxic challenge, which is reversed by the administration of a NOS inhibitor. Thus, it has been proposed that ADH3 activity provides a mechanism to limit the impact of RNS on cells and tissues by promoting the decomposition of GSNO. It follows that GSH and GSNO would play a central role in determining intracellular levels of protein RSNOs (74). This is supported by results obtained from pharmacological manipulations targeted at modulating intracellular GSH. Pretreatment of cells with L-buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthetase, or the glucose-6-phosphate dehydrogenase (G6PDH) inhibitor dehydroepiandrosterone (DHEA) both increase HMW RSNOs (36). In the case of DHEA, the decrease in GSH is thought to be the result of diminished NADPH availability upon G6PDH inhibition and consequential inactivation of the NADPH-dependent glutathione reductase.

Many signaling proteins rely on a network of redox-sensitive enzymes to regulate their activity by changing the oxidation state of key thiols. These redox-sensitive enzymes include the glutathioredoxin and thioredoxin systems, which regulate various key signaling components and transcription

factors. Whether the activity of these systems influences protein nitrosation is unclear, although studies that examined the interaction between NO and thioredoxin would point toward this direction (20, 35). There are also indications that, at least *in vitro*, a variety of intracellular enzymes, including xanthine oxidase (110), glutathione peroxidase (25), and Cu,Zn-SOD (51), mediate the decomposition of RSNOs. Undoubtedly, molecular strategies allowing for the specific down-regulation of proteins represent important approaches to delineate the relationship between thiol metabolism, intracellular redox state, and RSNO formation and decomposition *in vivo*.

PROTEIN NITROSATION: GENERAL CONCEPTS

Nitrosation reactions are not restricted to cysteine residues, and tryptophan can also form stable nitroso species (114). The chemical pathways underlying nitrosation of tryptophan by physiologically relevant concentrations of NO are not well characterized, and cysteine and tryptophan may in fact compete for the same nitrosating species. In accordance, Harohalli *et al.* demonstrated that Trp214 is the primary site of nitrosation in human serum albumin such that mutation of this residue resulted in an increase in Cys34 nitrosation (37). Peptides and proteins containing nitrosated tryptophan residues have demonstrated biological activities, including vasorelaxation and antiplatelet activity (128). This is in spite of the inability of *S*-nitrosotryptophan to release free NO spontaneously, suggesting that tryptophan and cysteine may exchange NO equivalents through transnitrosation reactions.

In addition to amino acid selectivity, Stamler *et al.* identified putative consensus nitrosation motifs in proteins that may be contained within the primary sequence or the three-dimensional arrangement of the protein (106). In this motif, the location of a cysteine residue between a basic and acidic residue may target the sulfhydryl group for nitrosation by increasing its nucleophilicity (*i.e.,* by decreasing the pK_a of the thiol group). Studies by Nudler and co-workers also suggest that the hydrophobic interior of proteins accelerates the formation of nitrosating species, including N_2O_3, via the accumulation of NO and O_2 (89). Proteins may serve as catalysts of their own nitrosation through a micellar mechanism similar to the increase in NO autoxidation observed in the interior of membrane bilayers. In accordance, the nitrosation of albumin was found to be more efficient than that of GSH and cysteine in the presence of authentic NO, and disruption of the protein core through protease treatment indicated abrupt changes in NO reactivity (97). Setting apart the potential role of micellar nitrosation in vascular homeostasis (97), these studies would suggest that the hydrophobic regions of protein in cells provide a promiscuous environment for the formation and utilization of RNS. Lastly, steric considerations limiting the accessibility of protein thiols to LMW RSNOs and/or to certain RNS may also decide the nature of the modification, such as oxidation, thiolation, and nitrosation. Thus, protein nitrosation is a specific process targeting only a restricted number of proteins and limited to key cysteine residues within these proteins.

PROTEIN NITROSATION AS A REGULATOR OF CELL SIGNALING

The literature regarding the modulation of cell signaling by thiol nitrosation is expanding rapidly, and it is beyond the present work to review this body of literature in an exhaustive manner. Rather, we will review two prototypical examples to outline the scope and limitations of the literature suggesting a relation between thiol nitrosation and cell signaling.

Ras and NO

The $p21^{Ras}$ (Ras) subfamily of small, monomeric GTPases is involved in the regulation of growth and differentiation, in contrast to the Rho/Rac subfamily, which generally controls cytoskeletal rearrangement. Ras GTPase activity is enhanced by guanine nucleotide exchange factors (GEFs), which substitute GDP bound to Ras in its inactive state with GTP, and GTPase-activating proteins (GAPs), which hydrolyze the bound GTP to GDP, inactivating Ras (40). In the mid-1990s, NO was recognized as a critical signaling mediator in the immune system (64, 65), and as Ras was known to play a role in T-cell activation by mitogens (7, 15), it was hypothesized to be a target of modulation by NO. Lander and co-workers found that incubation of Jurkat T cells with NO gas enhanced guanine nucleotide exchange on immunoprecipitated Ras, and this enhancement was attributed to RSNO formation in the GEF binding region, because an antibody that specifically binds in this region also blocked RSNO formation (66). The same group later identified Cys118 as the nitrosated residue using mass spectrometry (68). A mutant form of Ras was generated in which Cys118 was mutated to Ser (termed $p21^{Ras}$C118S), and this mutant was resistant to NO activation *in vitro* and in cells while retaining normal basal and GEF-stimulated activation kinetics (68). The Cys118 residue is remarkably conserved throughout species, and the Ras family members Ha-, Ki-, and N-Ras, rap1A, rap1B, rab1, and rab3 also contain a Cys residue in the GEF binding region, whereas ral, tc21, R-Ras, rap2, and rho do not (68).

The mechanism by which nitrosation activates Ras is still under investigation. It was hypothesized that nitrosation induced a conformational change in the protein (66) or that nitrosation resulted in altered affinity for the guanine nucleotide (68). However, recent nuclear magnetic resonance studies refute these hypotheses, showing that stably nitrosated Ras has a similar structure to nonnitrosated Ras, and that nitrosated Ras has similar guanine nucleotide dissociation rates as nonnitrosated Ras (115). These studies suggest that the chemical process of nitrosation was responsible for Ras activation by NO, possibly via the formation of a thiyl radical on Ras (39, 115). In spite of these advances in structural biology and biochemistry, it is still unclear precisely how NO induces Ras activity.

Recently, proteomic and genomic approaches have identified Ras as a major target of nitrosation in physiological models of NO production, including endothelial shear stress and neuronal signaling in the brain (43, 48). The recently discovered Ras family member Dexras (for dexamethasone-inducible Ras) is a nitrosated protein enriched in the brain,

discovered by yeast two-hybrid screening of a hippocampal/cortical rat brain cDNA library with the phosphotyrosine binding domain of CAPON [C-terminal PDZ domain ligand of neuronal NOS (nNOS)], a protein that complexes with nNOS. The authors demonstrate that activation of Dexras, but not H-Ras, is blocked by deletion of nNOS and that Dexras forms a complex with nNOS and CAPON *in vivo* (18). In spite of the diffusibility of NO, the authors suggest that specific signaling through Dexras is achieved by colocalization with NOS. However, there is little evidence for the consequence of Ras nitrosation given in these studies.

Indeed, there have been only a few examples in the published literature of relevant downstream consequences of Ras nitrosation. Lander and co-workers have described several pathways activated by NO in neuronal PC12 cells, Jurkat T cells, and human umbilical vein endothelial cells (68). Early work described activation of nuclear factor-*k*B by NO in PC12 cells, which was blocked by the NO-nonresponsive Ras mutant $p21^{Ras}C118S$ (68). This was followed up in a subsequent article in which the authors demonstrated that cells expressing the mutant $p21^{Ras}C118S$ failed to sustain basal phosphoinositide 3-kinase (PI3K) activity over long-term neuronal growth factor stimulation, leading to spontaneous apoptosis. These observations indicated that NO production in response to nerve growth factor (NGF) leads to cell survival through the Ras/PI3K interaction. However, these cells had normal differentiation characteristics and mitogen-activated protein (MAP) kinase activity, indicating those pathways are NO-independent in spite of being Ras-dependent in NGF stimulation (108). Another study by the same group characterized the response of the MAP kinases extracellular regulated kinase (ERK), p38, and c-Jun NH_2-terminal kinase, to NO in Jurkat T cells, and found that all three pathways were inhibited in the presence of a farnesyl transferase inhibitor, indicating the involvement of a farnesylated small GTPase (67). Finally, NO activation of Ras was demonstrated to be involved in ischemic preconditioning of neurons by activation of the ERK pathway. Exposure of cells to short nonlethal ischemic events protects them against longer ischemic insult, and protection was reversed by NOS inhibitors, overexpression of a dominant negative Ras, Raf, MEK, and ERK, but not PI3K (30).

There remain many questions about the relevance of Ras nitrosation on downstream signaling. The NO-nonresponsive mutant $p21^{Ras}C118S$ holds great potential for differentiating between nitrosation-dependent and -independent Ras signaling in a number of cell systems. However, until the mechanism of Ras activation by nitrosation is understood, the effects of this mutant on cell signaling will remain unclear. The possibility remains that the effects of NO on Ras activation is not, in fact, due to nitrosation, but to the formation of a thiyl radical on Ras or another intermediate that may also be generated by exposure to reactive oxygen species, and that nitrosation of Ras is only a by-product of exposure to NO. However, nitrosation of Ras remains a convenient explanation of many cyclic GMP-independent effects of NO on cell signaling.

Caspases, nitrosation, and denitrosation

The cysteine-containing aspartate-specific proteases (caspases) play an essential role in regulating apoptosis (10). There are at least 14 isoforms identified to date, which consist of three subfamilies: (a) the interleukin-1β-converting enzyme (ICE) subfamily, comprising caspases 1, 4, and 5; (b) the CED-3 (*Caenorhabditis elegans* cell death protein 3)/CPP32 (cysteine protease 32) subfamily, comprising caspases 3, 6, 7, 8, 9, and 10; and (c) the ICH-1 (ice and ced-3 homologue)/NEDD2 (neural precursor cell expressed, developmentally down-regulated 2) subfamily, comprising caspase-2. In general terms, caspases are synthesized as inactive proenzymes, which are activated following cleavage at specific aspartate sites. Caspases 8, 9, and 10 are initiator caspases that transduce apoptic signals by directly activating the downstream executioner caspases 3, 6, and 7. Recent evidence suggests that the activity of these proteins is inhibited in cell-free systems upon nitrosation of specific cysteine residues and that, in certain cell types, nitrosation reactions may affect the activity, the association, and the cellular distribution of caspases. It is the only example suggesting that the removal of NO (or NO^+) from cysteine residues—or denitrosation—impacts a signal transduction pathway.

In 1997, three groups determined that caspase-3 activity is inhibited in cells upon exposure to NO produced either exogenously by addition of an NO donor or endogenously upon activation of NOS (14, 59, 85). Because the active site of caspase-3 located in the p17 subunit contains a functionally important cysteine residue (Cys163), it became evident that the activity of this enzyme might be inhibited upon nitrosation of this cysteine. In the study of Dimmeler *et al.,* the bacterially expressed wild-type and Cys163-mutated p17 subunits were purified (14). NO generated by sodium nitrite or sodium nitroprusside time-dependently nitrosated one thiol group per p17 subunit and led to a complete inhibition of enzyme activity *in vitro.* When Cys163 was mutated to Ala163, no reactive SH group was detectable, suggesting that Cys163 was the main nitrosation site. Similar strategies were used in the other studies to support an association between caspase-3 nitrosation, its inactivation, and inhibition of apoptosis. Evidence for nitrosation was based on the reversibility of the modification by reducing agents such as dithiothreitol or sensitivity to mercury treatment because this metal decomposes RSNOs. Direct evidence that caspase-3 could be indeed nitrosated in cells came later when Rössig and co-workers utilized electron spin resonance combined with immunoprecipitation of overexpressed Myc-tagged p17 to identify the nitrosated subunit at Cys163 (100).

Although the aforementioned studies clearly established a relationship between apoptosis, caspase-3 activity, and RNS, the direct implications of NO-mediated reactions on caspase-3 structure and activity are unclear. Zech and co-workers characterized NO-modified caspase-3 *in vitro* using mass spectrometry (121). This study revealed that NO treatment leads to a variety of modifications in addition to RSNO that include oxidation and mixed disulfide formation if GSH is present. Although the significance of the oxidative modifications was not examined, this study confirmed that Cys163 in the p17 subunit is a primary site of nitrosation. Most interestingly, this site is resistant to transnitrosation reactions with physiologically relevant concentrations of GSH, indicating that stable protein RSNOs might be formed in cells even in the presence of large amounts of GSH. This is further sup-

ported by the observation that NOS inhibition in cells does not significantly decrease caspase-3 nitrosation at least for the first 2 h (80). Overall, it is difficult to speculate as to the actual structural impact of nitrosation because it is not associated with major changes, such as dissociation of the caspase-3 multimer (121).

In addition to caspase-3, the activities of six other caspases are inhibited *in vitro,* including upstream mediators such as caspases 8 and 9 (72). Caspase-8 activity in tumor necrosis factor-α (TNF-α)/actinomycin D-treated cultured hepatocytes is inhibited upon iNOS induction with a resultant decrease in the activation of downstream caspases 3 and 9 (60). In an *in vitro* reconstitution system, the same authors showed that nitrosation of recombinant caspase-8 can indeed inhibit the downstream cleavage of procaspases 9 and 3. Thus, NO may acutely regulate apoptosis through direct nitrosation of active upstream and downstream caspases. However, there is now evidence to suggest that procaspases are also nitrosated in cells and that denitrosation occurs upon cleavage of the different zymogens to the active caspase. Mannick and co-workers directly demonstrated that nitrosation of procaspase-3 in Jurkat cells expressing iNOS and denitrosation upon Fas activation are associated with proteolytic activation of caspase-3 (80). In a fashion similar to that of caspase-3, procaspase-9 nitrosation in cells is diminished by activation of the TNF-α receptor (58). In cell-free systems, caspases 3 and 9 are maintained in their inactive zymogen form by NO treatment, an effect reversed by reduction with dithiothreitol (109). This indicates that NO-mediated nitrosation of caspases 3 and 9 contributes to maintain these molecules in their inactive form. Whether nitrosation of procaspase occurs in the active site is still to be demonstrated.

The mechanism(s) of procaspase denitrosation and how this would modulate proteolytic cleavage are unknown. The reported stability of nitrosated Cys163 in the active site of caspase-3 and the fact that the majority of nitrosated caspase localizes in the mitochondria suggest mechanisms of nitrosation/denitrosation specific to caspases and to the mitochodrial environment (81). Zeigler and co-workers reported a differential sensitivity of caspases to NO donors and LMW RSNOs, and they indicated that there might be important differences in terms of protein nitrosation between these two approaches (123). This is not, however, clearly supported by the existing literature. There are also strong indications that some of the effects associated with NO-mediated nitrosation are also incorrectly interpreted because in some cases reducing agents do not fully restore caspase activity (122). Of course, the role of NO in modulating apoptosis is not restricted to nitrosation of specific residues in caspases. Clearly, understanding the impact of nitrosation and other associated reactions on NO-mediated modulation of the apoptotic signal will require the integration of multiple effects associated with NO production, including changes in protein structure and activity, protein–protein interactions, and localization.

CONCLUSION

This review described some of the evidence in support of the relevance of thiol nitrosation in cell signaling. An important question is whether the general principles that dictate nitrosation *in vitro* are applicable to the *in vivo* setting. This is only beginning to be examined experimentally in cell systems and *in vivo,* unraveling important new paradigms such as the role of the GSH and thioredoxin systems in regulating protein nitrosation. Independent of the mechanisms of nitrosation, the studies outlined in this review would suggest that nitrosation by NOS-derived NO provides a general form of signaling that is used in many different conditions in a fashion similar to NADPH oxidase-derived $O_2^{\cdot -}$ and hydrogen peroxide (88). An important challenge will be to identify the specificity of the nitrosative versus oxidative pathways because both chemistries seem to be overlapping at least *in vitro.* Recent advances in proteomics allowing for the detection of specific modifications, such as nitration, oxidation, thiolation, and nitrosation, will also provide the means to decipher NO-mediated reactions at the level of specific proteins and to shift the bulk of the research regarding nitrosation pathways from the *in vitro* to the *in vivo* setting.

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ABBREVIATIONS

ADH3; alcohol dehydrogenase 3; Dexras, dexamethasone-inducible ras; DHEA, dehydroepiandrosterone; ERK, extracellular regulated kinase; GEF, guanine nucleotide exchange factor; GSH, glutathione; GSNO, *S*-nitrosoglutathione; GSSG, glutathione disulfide; HMW, high molecular weight; HNO, nitroxyl; HNO_2, nitrous acid; HONNOH, hyponitrous acid; iNOS, inducible nitric oxide synthase; LMW, low molecular weight; MAP, mitogen-activated protein; NGF, nerve growth factor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NO^+, nitrosonium; NO_2, nitrogen dioxide; NO_2^-, nitrite; NO_3^-, nitrate; N_2O, nitrous oxide; N_2O_3, dinitrogen trioxide; NOS, nitric oxide synthase; $O_2^{\bullet -}$, superoxide; $OONO^-$/ONOOH, peroxynitrite; PI3K, phosphoinositide 3-kinase; Raf, raf protein; Ras, ras protein; RNNO, *N*-nitrosamine; RNS, reactive nitrogen species; RSNO, nitrosothiol; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α.

REFERENCES

1. Aravindakumar CT, De Ley M, and Ceulemans J. Kinetics of the anaerobic reaction of nitric oxide with cysteine, glutathione and cysteine-containing proteins: implications for in vivo *S*-nitrosation. *J Chem Soc Perkin Trans 2* 663–669, 2002.
2. Balazy M, Kaminski PM, Mao K, Tan J, and Wolin MS. *S*-Nitroglutathione, a product of the reaction between

peroxynitrite and glutathione that generates nitric oxide. *J Biol Chem* 273: 32009–32015, 1998.
3. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite; the good, the bad, and the ugly. *Am J Physiol* 271: C1424–C1437, 1996.
4. Beckman JS, Beckman TW, Chen J, Marshall PA, and Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci U S A* 87: 1620–1624, 1990.
5. Boese M, Mordvintcev PI, Vanin AF, Busse R, and Mülsch A. *S*-Nitrosation of serum albumin by dinitrosyliron complex. *J Biol Chem* 270: 29244–29249, 1995.
6. Bryan NS, Rassaf T, Maloney RE, Rodriguez CM, Saijo F, Rodriguez JR, and Feelisch M. Cellular targets and mechanisms of nitros(yl)ation: an insight into their nature and kinetics in vivo. *Proc Natl Acad Sci U S A* 101: 4308–4313, 2004.
7. Cantrell DA, Graves JD, Izquierdo M, Lucas S, and Downward J. Lymphocyte-T activation signals. *Ciba Found Symp* 164: 208–222, 1992.
8. Caulfield JL, Singh SP, Wishnok JS, Deen WM, and Tannenbaum SR. Bicarbonate inhibits *N*-nitrosation in oxygenated nitric oxide solutions. *J Biol Chem* 271: 25859–25863, 1996.
9. Chen B and Deen WM. Analysis of the effects of cell spacing and liquid depth on nitric oxide and its oxidation products in cell culture. *Chem Res Toxicol* 14: 135–147, 2001.
10. Chung HT, Pae HO, Choi BM, Billiar TR, and Kim YM. Nitric oxide as a bioregulator of apoptosis. *Biochem Biophys Res Commun* 282: 1075–1079, 2001.
11. Clancy RM, Levartovsky D, Leszczynska-Pziak J, Yegudin J, and Abramson SB. Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: evidence for *S*-nitrosoglutathione as a bioreactive intermediary. *Proc Natl Acad Sci U S A* 91: 3680–3684, 1994.
12. Dicks AP and Williams DL. Generation of nitric oxide from *S*-nitrosothiols using protein-bound Cu^{+2} sources. *Chem Biol* 3: 655–659, 1996.
13. Dicks AP, Swift HR, Williams DLH, Butler AR, Al-Sa'doni HH, and Cox BG. Identification of Cu^{+} as the effective reagent in nitric oxide formation from *S*-nitrosothiols (RSNO). *J Chem Soc Perkin Trans* 2 481–487, 1996.
14. Dimmeler S, Haendeler J, Nehls M, and Zeiher AM. Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J Exp Med* 185: 601–607, 1997.
15. Downward J, Graves JD, Warne PH, Rayter S, and Cantrell DA. Stimulation of p21Ras upon T-cell activation. *Nature* 346: 719–723, 1990.
16. Espey MG, Miranda KM, Thomas DD, and Wink DA. Distinction between nitrosating mechanisms within human cells and aqueous solution. *J Biol Chem* 276: 30085–30091, 2001.
17. Eu JP, Liu L, Zeng M, and Stamler JS. An apoptotic model for nitrosative stress. *Biochemistry* 39: 1040–1047, 2000.
18. Fang M, Jaffrey SR, Sawa A, Ye KQ, Luo XJ, and Snyder SH. Dexras1: a G protein specifically coupled to neuronal nitric oxide synthase via CAPON. *Neuron* 28: 183–193, 2000.
19. Feelisch M, Rassaf T, Mnaimneh S, Singh N, Bryan NS, Jourd'heuil D, and Kelm M. Concomitant *S*-, *N*-, and heme-nitros(yl)ation in biological tissues and fluids: implications for the fate of NO in vivo. *FASEB J* 16: 1775–1785, 2002.
20. Ferret PJ, Soum E, Negre O, Wollman EE, and Fradelizi D. Protective effect of thioredoxin upon NO-mediated cell injury in THP1 monocytic human cells. *Biochem J* 346 Pt 3: 759–765, 2000.
21. Ford E, Hughes MN, and Wardman P. Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH. *Free Radic Biol Med* 32: 1314–1323, 2002.
22. Foster MW and Stamler JS. New insights into protein *S*-nitrosylation: mitochondria as a model system. *J Biol Chem* 279: 25891–25897, 2004.
23. Foster MW, McMahon TJ, and Stamler JS. *S*-Nitrosylation in health and disease. *Trends Mol Med* 9: 160–168, 2003.
24. Fox-Robichaud A, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, and Kubes P. Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *J Clin Invest* 101: 2497–2505, 1998.
25. Freedman JE, Frei B, Welch GN, and Loscalzo J. Glutathione peroxidase potentiates the inhibition of platelet function by *S*-nitrosothiols. *J Clin Invest* 96: 394–400, 1995.
26. Gladwin MT, Ognibene FP, Pannel LK, Nichols JS, Pease-Fye ME, Shelhamer JH, and Schechter AN. Relative role of heme nitrosylation and β-cysteine 93 nitrosation in the transport and metabolism of nitric oxide by hemoglobin in the human circulation. *Proc Natl Acad Sci U S A* 97: 9943–9948, 2000.
27. Gladwin MT, Shelhamer JH, Schechter AN, Pease-Fye ME, Waclawiw MA, Panza JA, Ognibene FP, and Cannon RO 3rd. Role of circulating nitrite and *S*-nitrosohemoglobin in the regulation of regional blood flow in humans. *Proc Natl Acad Sci U S A* 97: 11482–11487, 2000.
28. Goldstein S and Czapski G. Mechanism of the nitrosation of thiols and amines by oxygenated NO solutions: the nature of the nitrosating intermediates. *J Am Chem Soc* 118: 3419–3425, 1996.
29. Goldstein S, Czapski G, Lind J, and Merényi G. Tyrosine nitration by simultaneous generation of NO and O_2^- under physiological conditions. *J Biol Chem* 275: 3031–3036, 2000.
30. Gonzalez-Zulueta M, Feldman AB, Klesse LJ, Kalb RG, Dillman JF, Parada LF, Dawson TM, and Dawson VL. Requirement for nitric oxide activation of p21(ras)/extracellular regulated kinase in neuronal ischemic preconditioning. *Proc Natl Acad Sci U S A* 97: 436–441, 2000.
31. Gow AJ and Stamler JS. Reactions between nitric oxide and haemoglobin under physiological conditions. *Nature* 391: 169–173, 1998.

32. Gow AJ, Buerk DG, and Ischiropoulos H. A novel reaction mechanism for the formation of *S*-nitrosothiol in vivo. *J Biol Chem* 272: 2841–2845, 1997.
33. Guo FH, De Raeve HR, Rice TW, Stuehr DJ, Thunnissen FBJM, and Erzurum SC. Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. *Proc Natl Acad Sci U S A* 92: 7809–7813, 1995.
34. Hackam DJ, Rotstein OD, Zhang WJ, Demaurex N, Woodside M, Tsai O, and Grinstein S. Regulation of phagosomal acidification. Differential targeting of Na^+/H^+ exchangers, Na^+/K^+-ATPases, and vacuolar-type H^+-ATPases. *J Biol Chem* 272: 29810–29820, 1997.
35. Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM, and Dimmeler S. Redox regulatory and anti-apoptotic functions of thioredoxin depend on *S*-nitrosylation at cysteine 69. *Nat Cell Biol* 4: 743–749, 2002.
36. Haqqani AS, Do SK, and Birnboim HC. The role of a formaldehyde dehydrogenase-glutathione pathway in protein *S*-nitrosation in mammalian cells. *Nitric Oxide* 9: 172–181, 2003.
37. Harohalli K, Petersen CE, Ha CE, Feix JB, and Bhagavan NV. Site-directed mutagenesis studies of human serum albumin define tryptophan at amino acid position 214 as the principal site for nitrosation. *J Biomed Sci* 9: 47–58, 2002.
38. Hedberg JJ, Griffiths WJ, Nilsson SJF, and Hoog JO. Reduction of *S*-nitrosoglutathione by human alcohol dehydrogenase 3 is an irreversible reaction as analysed by electrospray mass spectrometry. *Eur J Biochem* 270: 1249–1256, 2003.
39. Heo JY and Campbell SL. Mechanism of p21(Ras) *S*-nitrosylation and kinetics of nitric oxide-mediated guanine nucleotide exchange. *Biochemistry* 43: 2314–2322, 2004.
40. Hingorani SR and Tuveson DA. Ras redux: rethinking how and where Ras acts. *Curr Opin Genet Dev* 13: 6–13, 2003.
41. Hodges GR, Marwaha J, Paul T, and Ingold KU. A novel procedure for generating both nitric oxide and superoxide in situ from chemical sources at any chosen mole ratio. First application: tyrosine oxidation and a comparison with preformed peroxynitrite. *Chem Res Toxicol* 13: 1287–1293, 2000.
42. Hoffmann J, Haendeler J, Zeiher AM, and Dimmeler S. TNFα and oxLDL reduce protein *S*-nitrosylation in endothelial cells. *J Biol Chem* 44: 41383–41387, 2001.
43. Hoffmann J, Dimmeler S, and Haendeler J. Shear stress increases the amount of *S*-nitrosylated molecules in endothelial cells: important role for signal transduction. *FEBS Lett* 551: 153–158, 2003.
44. Hogg N. The kinetics of *S*-transnitrosation—a reversible second-order reaction. *Anal Biochem* 272: 257–262, 1999.
45. Hogg N, Singh RJ, and Kalyanaraman B. The role of glutathione in the transport and catabolism of nitric oxide. *FEBS Lett* 382: 223–228, 1996.
46. Hunt J and Gaston B. Airway nitrogen oxide measurements in asthma and other pediatric respiratory diseases. *J Pediatr* 137: 1–12, 2000.
47. Inoue K, Akaike T, Miyamoto Y, Okamoto T, Sawa T, Otagiri M, Suzuki S, Yoshimura T, and Maeda H. Nitrosothiol formation catalyzed by ceruloplasmin. *J Biol Chem* 274: 27069–27075, 1999.
48. Jaffrey SR, Erdjument-Bromage H, Ferris CD, Tempst P, and Snyder SH. Protein *S*-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat Cell Biol* 3: 193–197, 2001.
49. Jensen DE, Belka GK, and Du Bois GC. *S*-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. *Biochem J* 331: 659–668, 2004.
50. Jones DP. Intracellular diffusion gradients of O_2 and ATP. *Am J Physiol* 86: 663–675, 1986.
51. Jourd'heuil D, Laroux SF, Miles AM, Wink DA, and Grisham MB. Effect of superoxide dismutase on the stability of *S*-nitrosothiols. *Arch Biochem Biophys* 361: 323– 330, 1999.
52. Jourd'heuil D, Miranda KM, Kim MS, Espey MG, Vodovotz Y, Laroux S, Mai CT, Miles AM, Grisham MB, and Wink DA. The oxidative and nitrosative chemistry of the nitric oxide/superoxide reaction in the presence of bicarbonate. *Arch Biochem Biophys* 365: 92–100, 1999.
53. Jourd'heuil D, Gray L, and Grisham MB. *S*-Nitrosothiol formation in blood of lipopolysaccharide-treated rats. *Biochem Biophys Res Commun* 273: 22–25, 2000.
54. Jourd'heuil D, Hallén K, Feelisch M, and Grisham MB. Dynamic state of *S*-nitrosothiols in human plasma and whole blood. *Free Radic Biol Med* 28: 409–417, 2000.
55. Jourd'heuil D, Jourd'heuil FL, Kutchukian PS, Musah RA, Wink DA, and Grisham MB. Reaction of superoxide and nitric oxide with peroxynitrite. *J Biol Chem* 276: 28799–28805, 2001.
56. Jourd'heuil D, Jourd'heuil FL, and Feelisch M. Oxidation and nitrosation of thiols at low micromolar exposure to nitric oxide. Evidence for a free radical mechanism. *J Biol Chem* 278: 15720–15726, 2003.
57. Keshive M, Singh S, Wishnok JS, Tannenbaum SR, and Deen WM. Kinetics of *S*-nitrosation of thiols in nitric oxide solutions. *Chem Res Toxicol* 9: 988–993, 1996.
58. Kim JE and Tannenbaum SR. *S*-Nitrosation regulates the activation of endogenous procaspase-9 in HT-29 human colon carcinoma cells. *J Biol Chem* 279: 9758–9764, 2004.
59. Kim Y-M, Talanian RV, and Billiar TR. Nitric oxide inhibits apoptosis by preventing increase in caspase-3-like activity via two distinct mechanisms. *J Biol Chem* 272: 31138–31148, 1997.
60. Kim YM, Kim TH, Chung HT, Talanian RV, Yin XM, and Billiar TR. Nitric oxide prevents tumor necrosis factor alpha-induced rat hepatocyte apoptosis by the interruption of mitochondrial apoptotic signaling through *S*-nitrosylation of caspase-8. *Hepatology* 32: 770–778, 2000.
61. Kim Y-M, Chung H-T, Simmons RL, and Billiar TR. Cellular non-heme iron content is a determinant of nitric oxide-mediated apoptosis, necrosis, and caspase inhibition. *J Biol Chem* 275: 10954–10961, 2000.
62. Klebanoff SJ. Reactive nitrogen intermediates and antimicrobial activity: role of nitrite. *Free Radic Biol Med* 14: 351–360, 1993.

63. Kosaka H, Wishnok JS, Miwa M, Leaf CD, and Tannenbaum SR. Nitrosation by stimulated macrophages. Inhibitors, enhancers and substrates. *Carcinogenesis* 10: 563–566, 1989.
64. Lander HM, Sehajpal P, Levine DM, and Novogrodsky A. Activation of human peripheral-blood mononuclear-cells by nitric oxide-generating compounds. *J Immunol* 150: 1509–1516, 1993.
65. Lander HM, Sehajpal PK, and Novogrodsky A. Nitric-oxide signaling—a possible role for G-proteins. *J Immunol* 151: 7182–7187, 1993.
66. Lander MH, Ogiste JS, Pearce SFA, Levi R, and Novogrodsky A. Nitric oxide-stimulated guanine nucleotide exchange on p21ras. *J Biol Chem* 270: 7017–7020, 1995.
67. Lander HM, Jacovina AT, Davis RJ, and Tauras JM. Differential activation of mitogen-activated protein kinases by nitric oxide-related species. *J Biol Chem* 271: 19705–19709, 1996.
68. Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, and Quilliam LA. A molecular redox switch on p21(ras)—structural basis for the nitric oxide-p21(ras) interaction. *J Biol Chem* 272: 4323–4326, 1997.
69. Lane P, Hao G, and Gross SS. *S*-Nitrosylation is emerging as a specific and fundamental posttranslational protein modification: head-to-head comparison with *O*-phosphorylation. *Sci STKE* 2001: RE1, 2001.
70. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, and Kelm M. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci U S A* 98: 12814–12819, 2001.
71. Lewis R, Tannenbaum SR, and Deen WM. Kinetics of *N*-nitrosation in oxygenated nitric oxide solutions at physiological pH: role of nitrous anhydride and effects of phosphate and chloride. *J Am Chem Soc* 117: 3933–3939, 1995.
72. Li J, Billiar TR, Talanian RV, and Kim Y-M. Nitric oxide reversibly inhibits seven members of the caspase family via *S*-nitrosylation. *Biochem Biophys Res Commun* 240: 419–424, 1997.
73. Licht WR, Tannenbaum SR, and Deen WM. Use of ascorbic acid to inhibit nitrosation: kinetic and mass transfer consideration for an in vitro system. *Carcinogenesis* 9: 365–372, 1988.
74. Liu L, Hausladen A, Zeng M, Que L, Heitman J, and Stamler JS. A metabolic enzyme for *S*-nitrosothiol conserved from bacteria to humans. *Nature* 410: 490–494. 2001.
75. Liu L, Yan Y, Zeng M, Zhang J, Hanes MA, Ahearn G, McMahon TJ, Dickfeld T, Marshall HE, Que LG, and Stamler JS. Essential roles of *S*-nitrosothiols in vascular homeostasis and endotoxic shock. *Cell* 116: 617–628, 2004.
76. Liu X, Miller MJS, Joshi MS, Thomas DD, and Lancaster JR Jr. Accelerated reaction of nitric oxide with O_2 within the hydrophobic interior of biological membranes. *Proc Natl Acad Sci U S A* 95: 2175–2179, 1998.
77. Liu Z, Rudd MA, Freedman JE, and Loscalzo J. *S*-Transnitrosation reactions are involved in the metabolic fate and biological actions of nitric oxide. *J Pharmacol Exp Ther* 284: 526–534, 1998.
78. Mallis RJ and Thomas JA. Effect of *S*-nitrosothiols on cellular glutathione and reactive protein sulfhydryls. *Arch Biochem Biophys* 383: 60–69, 2000.
79. Mannick JB and Schonhoff CM. Nitrosylation: the next phosphorylation? *Arch Biochem Biophys* 408: 1–6, 2002.
80. Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, Kane LS, Gow AJ, and Stamler JS. Fas-induced caspase denitrosylation. *Science* 284: 651–654, 1999.
81. Mannick JB, Schonhoff C, Papeta N, Ghafourifar P, Szibor M, Fang K, and Gaston B. *S*-Nitrosylation of mitochondrial caspases. *J Cell Biol* 154: 1111–1116, 2001.
82. Meyer DJ, Kramer H, Ozer N, Coles B, and Ketterer B. Kinetics and equilibria of *S*-nitrosothiol–thiol exchange between glutathione, cysteine, penicillamines and serum-albumin. *FEBS Lett* 345: 177–180, 1994.
83. Miles AM, Bohle DS, Glassbrenner PA, Hansert B, Wink DA, and Grisham MB. Modulation of superoxide-dependent oxidation and hydroxylation reactions by nitric oxide. *J Biol Chem* 271: 40–47, 1996.
84. Mirvish SS. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 93: 17–48, 1995.
85. Mohr S, Zech B, Lapetina EG, and Brune B. Inhibition of caspase-3 by *S*-nitrosation and oxidation caused by nitric oxide. *Biochem Biophys Res Commun* 238: 387–391, 1997.
86. Moro MA, Darley-Usmar VM, Lizasoain I, Su Y, Knowles RG, Radomski MW, and Moncada S. The formation of nitric oxide donors from peroxynitrite. *Br J Pharmacol* 116: 1999–2004, 1995.
87. Nalwaya N and Deen WM. Analysis of cellular exposure to peroxynitrite in suspension cultures. *Chem Res Toxicol* 16: 920–932, 2003.
88. Nathan C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. *J Clin Invest* 111: 769–778, 2003.
89. Nedospasov A, Rafikov R, and Nudler E. An autocatalytic mechanism of protein nitrosylation. *Proc Natl Acad Sci U S A* 97: 13543–13548, 2000.
90. Ng ESM, Jourd'heuil D, McCord JM, Hernandez D, Yasui M, Knight D, and Kubes P. Enhanced *S*-nitroso-albumin formation from inhaled NO during ischemia/reperfusion. *Circ Res* 94: 559–565, 2004.
91. O'Donnell VB, Eiserich JP, Chumley PH, Jablonsky MJ, Krishna NR, Kirk M, Barnes S, Darley-Usmar VM, and Freeman BA. Nitration of unsaturated fatty acids by nitric oxide-derived reactive nitrogen species peroxynitrite, nitrous acid, nitrogen dioxide, and nitronium ion. *Chem Res Toxicol* 12: 83–92, 1999.
92. Padgett CM and Whorton AR. Cellular responses to nitric oxide: role of protein *S*-thiolation/dethiolation. *Arch Biochem Biophys* 358: 232–242, 1998.
93. Pfieffer S and Mayer B. Lack of tyrosine nitration by peroxynitrite generated at physiological pH. *J Biol Chem* 273: 27280–27285, 1998.

94. Pryor WA, Church DF, Govindan CK, and Crank G. Oxidation of thiols by nitric oxide and nitrogen dioxide: synthetic utility and toxicological implications. *J Org Chem* 47: 156–159, 1982.
95. Quijano C, Alvarez B, Gatti RM, Augusto O, and Radi R. Pathways of peroxynitrite oxidation of thiol groups. *Biochem J* 322: 167–173, 1997.
96. Radi R, Beckman JS, Bush KM, and Freeman BA. Peroxynitrite oxidation of sulfhydryls. *J Biol Chem* 266: 4244–4250, 1991.
97. Rafikova O, Rafikov R, and Nudler E. Catalysis of *S*-nitrosothiols formation by serum albumin: the mechanism and implication in vascular control. *Proc Natl Acad Sci U S A* 99: 5913–5918, 2002.
98. Ramachandran N, Root P, Jiang XM, Hogg PJ, and Mutus B. Mechanism of transfer of NO from extracellular *S*-nitrosothiols into the cytosol by cell-surface protein disulfide isomerase. *Proc Natl Acad Sci U S A* 98: 9539–9544, 2001.
99. Ricciardolo FLM, Gaston B, and Hunt J. Acid stress in the pathology of asthma. *J Allergy Clin Immunol* 113: 610–619, 2004.
100. Rössig L, Fichtlscherer B, Breitschopf K, Haendeler J, Zeiher AM, Mülsch A, and Dimmeler S. Nitric oxide inhibits caspase-3 by *S*-nitrosation in vivo. *J Biol Chem* 274: 6823–6826, 1999.
101. Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, and Freeman BA. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. *J Biol Chem* 269: 26066–26075, 1994.
102. Scharfstein JS, Keaney J, Slivka A, Welch GN, Vita JA, and Stamler JS. In vivo transfer of nitric oxide between a plasma protein-bound reservoir and low molecular weight thiols. *J Clin Invest* 94: 1432–1439, 1994.
103. Singh SP, Wishnok JS, Keshive M, Deen WM, and Tannenbaum SR. The chemistry of the *S*-nitrosoglutathione/glutathione system. *Proc Natl Acad Sci U S A* 93: 14428–14433, 1996.
104. Skinner KA, White CR, Patel R, Tan S, Barnes S, Kirk M, Darley-Usmar V, and Parks DA. Nitrosation of uric acid by peroxynitrite—formation of a vasoactive nitric oxide donor. *J Biol Chem* 273: 24491–24497, 1998.
105. Stamler JS, Jaraki O, Osborne JA, Simon DI, Keaney J, Vita J, Singel DJ, Valeri CR, and Loscalzo J. Nitric oxide circulates in mammalian plasma primarily as an *S*-nitroso adduct of serum albumin. *Proc Natl Acad Sci U S A* 89: 7674–7677, 1992.
106. Stamler JS, Toone EJ, Lipton SA, and Sucher NJ. (S)NO signals: translocation, regulation, and a consensus motif. *Neuron* 18: 691–696, 1997.
107. Stubauer G, Giuffrè A, and Sarti P. Mechanism of *S*-nitrosothiol formation and degradation mediated by copper ions. *J Biol Chem* 274: 28128–28133, 1999.
108. Teng KK, Esposito DK, Schwartz GD, Lander HM, and Hempstead BL. Activation of c-Ha-Ras by nitric oxide modulates survival responsiveness in neuronal PC12 cells. *J Biol Chem* 274: 37315–37320, 1999.
109. Torok NJ, Higuchi H, Bronk S, and Gores GJ. Nitric oxide inhibits apoptosis downstream of cytochrome *c* release by nitrosylating caspase 9. *Cancer Res* 62: 1648–1653, 2002.
110. Trujillo M, Alvarez MN, Peluffo G, Freeman BA, and Radi R. Xanthine oxidase-mediated decomposition of *S*-nitrosothiols. *J Biol Chem* 273: 7828–7834, 1999.
111. Van der Vliet A, Hoen PA, Wong PSY, Bast A, and Cross CE. Formation of *S*-nitrosothiols via direct nucleophilic nitrosation of thiols by peroxynitrite with elimination of hydrogen peroxide. *J Biol Chem* 273: 30255–30262, 1998.
112. Wade RS and Castro CE. Redox reactivity of iron(III) porphyrins and heme proteins with nitric oxide. Nitrosyl transfer to carbon, oxygen, nitrogen and sulfur. *Chem Res Toxicol* 3: 289–291, 1990.
113. Wardman P and von Sonntag C. Kinetic factors that control the fate of thiyl radicals in cells. *Methods Enzymol* 251: 31–45, 1995.
114. Williams DLH. *Nitrosation*. Cambridge: Press Syndicate of the University of Cambridge, 1988.
115. Williams JG, Pappu K, and Campbell SL. Structural and biochemical studies of p21Ras *S*-nitrosylation and nitric oxide-mediated guanine nucleotide exchange. *Proc Natl Acad Sci U S A* 100: 6376–6381, 2003.
116. Wink DA, Darbyshire JF, Nims RW, Saavedra JE, and Ford PC. Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: determination of the kinetics for oxidation and nitrosation by intermediates generated in the NO/O_2 reaction. *Chem Res Toxicol* 6: 23–27, 1993.
117. Wink DA, Grisham MB, Miles AM, Nims RW, Krishna M, Pacelli R, Teague D, Poore CMB, Cook JA, and Ford PC. Determination of selectivity of reactive nitrogen oxide species for various substrates. *Methods Enzymol* 268: 120–131, 1996.
118. Wink DA, Cook JA, Kim SY, Vodovotz Y, Pacelli R, Krishna MC, Russo A, Mitchell JB, Jourd'heuil D, Miles AM, and Grisham MB. Superoxide modulates the oxidation and nitrosation of thiols by nitric oxide-derived reactive intermediates. *J Biol Chem* 272: 11147–11151, 1997.
119. Wong PSY, Hyun J, Fukuto JM, Shirota FN, DeMaster EG, Shoeman DW, and Nagasawa HT. Reaction between *S*-nitrosothiols and thiols: generation of nitroxyl (HNO) and subsequent chemistry. *Biochemistry* 37: 5362–5371, 1998.
120. Zai A, Rudd MA, Scribner AW, and Loscalzo J. Cell-surface protein disulfide isomerase catalyzes transnitrosation and regulates intracellular transfer of nitric oxide. *J Clin Invest* 103: 393–399, 1999.
121. Zech B, Wilm M, van Eldik R, and Brüne B. Mass spectrometric analysis of nitric oxide-modified caspase. *J Biol Chem* 274: 20931–20936, 1999.
122. Zech B, Kohl R, von Knethen A, and Brune B. Nitric oxide donors inhibit formation of the Apaf-1/caspase-9 apoptosome and activation of caspases. *Biochem J* 371: 1055–1064, 2003.
123. Zeigler MM, Doseff AI, Galloway MF, Opalek JM, Nowicki PT, Zweier JL, Sen CK, and Marsh CB. Presentation of nitric oxide regulates monocyte survival through effects on caspase-9 and caspase-3 activation. *J Biol Chem* 278: 12894–12902, 2003.
124. Zhang H and Means GE. *S*-Nitrosation of serum albumin: spectrophotometric determination of its nitrosation

by simple *S*-nitrosothiols. *Anal Biochem* 237: 141–144, 1996.
125. Zhang H, Joseph J, Feix J, Hogg N, and Kalyanaraman B. Nitration and oxidation of hydrophobic tyrosine probe by peroxynitrite in membranes: comparison with nitration and oxidation of tyrosine by peroxynitrite in aqueous solution. *Biochemistry* 40: 7675–7686, 2001.
126. Zhang H, Bhargava K, Keszler A, Feix J, Hogg N, Joseph J, and Kalyanaraman B. Transmembrane nitration of hydrophobic tyrosyl peptides. Localization, characterization, mechanism of nitration, and biological implications. *J Biol Chem* 278: 8969–8978, 2003.
127. Zhang Y and Hogg N. Formation and stability of *S*-nitrosothiols in RAW 264.7 cells. *Am J Physiol Lung Cell Mol Physiol* 287: L467–L474, 2004.
128. Zhang YY, Xu AM, Nomen M, Walsh M, Keaney JF Jr, and Loscalzo J. Nitrosation of tryptophan residue(s) in serum albumin and model dipeptides. Biochemical characterization and bioactivity. *J Biol Chem* 271: 14271–14279, 1996.

Address reprint requests to:
David Jourd'heuil, Ph.D.
Albany Medical College
Center for Cardiovascular Sciences
47 New Scotland Avenue (MC8)
Albany, NY 12208

E-mail: jourdhd@mail.amc.edu

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3. Rajib Sengupta , Arne Holmgren . Thioredoxin and Thioredoxin Reductase in Relation to Reversible S-Nitrosylation. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. Rajib Sengupta, Arne Holmgren. 2012. The role of thioredoxin in the regulation of cellular processes by S-nitrosylation. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1820**:6, 689-700. [CrossRef]
5. Antonio Martínez-Ruiz, Susana Cadenas, Santiago Lamas. 2011. Nitric oxide signaling: Classical, less classical, and nonclassical mechanisms. *Free Radical Biology and Medicine* **51**:1, 17-29. [CrossRef]
6. M. V. Onufriev. 2010. Nitrosative stress in the brain: Autoantibodies to nitrotyrosine in the liquor as a potential marker. *Neurochemical Journal* **4**:3, 228-234. [CrossRef]
7. Mohamed Trebak , Roman Ginnan , Harold A. Singer , David Jourd'heuil . 2010. Interplay Between Calcium and Reactive Oxygen/Nitrogen Species: An Essential Paradigm for Vascular Smooth Muscle Signaling. *Antioxidants & Redox Signaling* **12**:5, 657-674. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
8. Moran Benhar, Michael T. Forrester, Jonathan S. Stamler. 2009. Protein denitrosylation: enzymatic mechanisms and cellular functions. *Nature Reviews Molecular Cell Biology* . [CrossRef]
9. John W. Semple, Edwin R. Speck, Antonio Fabron Jr, Rukhsana Aslam Michael Kim, John Freedman. 2008. A novel immunosuppressive pathway involving peroxynitrate-mediated nitration of platelet antigens within antigen-presenting cells. *Transfusion* **48**:9, 1917-1924. [CrossRef]
10. Jack R. Lancaster Jr.. 2008. Protein cysteine thiol nitrosation: Maker or marker of reactive nitrogen species-induced nonerythroid cellular signaling?. *Nitric Oxide* **19**:2, 68-72. [CrossRef]
11. Swati Basu, Xunde Wang, Mark T. Gladwin, Daniel B. Kim#ShapiroChemiluminescent Detection of S#Nitrosated Proteins: Comparison of Tri#iodide, Copper/CO/Cysteine, and Modified Copper/Cysteine Methods **440**, 137-156. [CrossRef]
12. Kenneth Hensley , Robert A. Floyd . 2005. Oxidative Modification of Proteins in Cell Signaling. *Antioxidants & Redox Signaling* **7**:5-6, 523-525. [Citation] [Full Text PDF] [Full Text PDF with Links]