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Forum Review

$^{\bullet}NO$, RSNO, $ONOO^{-}$, NO^{+}, $^{\bullet}NOO$, NOx—Dynamic Regulation of Oxidant Scavenging, Nitric Oxide Stores, and Cyclic GMP-Independent Cell Signaling

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ABSTRACT

Following its release from nitric oxide synthase, nitric oxide seldom perfuses the cytosol; rather this reactive mediator quickly interacts with available target molecules proximate to its site of release. Within the cell, virtually every component, low-molecular-weight oxidants and reductants, proteins, lipids, sugars, and nucleic acids can be modified by nitrogen oxides thus acting as potential targets for reactive nitrogen oxides. Adducts formed by nitrogen oxides often modulate the cellular activities of the target molecules, and these modified molecules may be differentially metabolized or localized. The formation of nitrogen oxide adducts can be a reversible process, and the reactive nitrogen species released may be specifically oxidized or reduced during the process. Recently, numerous studies have demonstrated that reversible nitration of cellular proteins acts to transduce molecular signals regulating such diverse processes as muscle contraction, neurotransmission, protein metabolism, and apoptosis. The vast numbers of molecules that undergo biologically relevant interactions with nitrogen oxides imply that the cellular concentration of nitrosated and nitrated species may effectively comprise a reserve or cellular store. Potentially, these nitroso reserves function as critical components of the overall redox status of the intracellular environs. Understanding the dynamic regulation of nitric oxide/nitrogen oxides release from these stores is likely to provide clues important in resolving the complex pathophysiology of poorly understood multifactorial disorders, including neurodegeneration, multiorgan failure, cardiomyopathy, and septic shock. Antioxid. Redox Signal. 3, 249–260.

INTRODUCTION

NITRIC OXIDE (NO) is well recognized as an intercellular messenger synthesized from the amino acid L-arginine by the NO synthase (NOS) family of oxidoreductases (17, 90). In biological systems, NO mediates a wide range of physiological processes including vasodilatation, tumor cell cytostasis, antimicrobial defense, neurotransmission, and bronchodilation (48, 93, 94). Induction of the high-output cytokine inducible isoform of NO is important in the latter processes, tumor cell cytostasis and antimicrobial defense. In these processes, the association of NO with protein heme or catalytic radical prosthetic groups can result in the inhibition of critical cellular enzymes including aconitase and ribonucleotide reductase (28, 29). In addition, NO can interact with DNA or membrane lipids resulting in adduct formation and lipid peroxidation. The accumulated NO-mediated damage to DNA, lipids, and/or proteins results, in turn, in inhibition of proliferation and microbial cell death (45, 105).

Intercellular signaling by NO was first characterized in the vasculature. In this process, soluble guanylate cyclase in vascular smooth muscle is activated through interaction of NO,

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produced by the endothelial-type isoform of NOS (NOS3), and released from adjacent endothelial cells, with protein-bound heme prosthetic groups (34, 92). Cyclic cGMP produced by the active cyclase subsequently associates with cyclic GMP-dependent kinases and phosphatases, altering their activity and initiating a cascade of events ultimately regulating muscle relaxation (34, 136). In a similar manner, NO produced in neuronal cells by the neuronal isoform of NOS (NOS1) modulates the synaptic activity of NO-sensitive neurons (18, 113).

Recently, numerous studies have demonstrated that the activities of an array of cellular components are reversibly and/or irreversibly regulated by nitrosation. In nitrosation reactions, nitrogen monooxide adducts are formed on available oxygen, nitrogen or thiol residues. In biological systems, the interaction of NO with molecular oxygen leads to the production of the facile nitrating species N_2O_3 via nitrogen dioxide radical ($^{\bullet}NO_2$) intermediates. However, peroxynitrite ($ONOO^-$), derived from the interaction of NO and superoxide ($O_2^{\bullet -}$), is also a potent nitrosating reagent (73, 94). In addition, in tissues nitrogen oxides (NOx) may interact reversibly and/or irreversibly with iron or iron/sulfur centers in prosthetic groups, sugars, lipids, and nucleic acids. An intriguing concept, derived from emerging information concerning the vast numbers of molecules that undergo biologically relevant interactions with NO, is that the cellular concentration of NOx is a critical component of its overall redox status (31, 73, 117). The great numbers of molecular interactions that can effectively sequester NO in a reversible manner imply the presence of intra- and extracellular NO stores. Understanding the dynamic regulation of NO/NOx release from these stores is likely to provide clues important in resolving the complex pathophysiology of poorly understood multifactorial disorders including neurodegeneration and septic shock.

CELLULAR SOURCES OF NOx AND NITROSATING SPECIES

In cells, NO is produced in response to inflammatory stimuli as a result of the up-regulation of the inducible form of NOS (NOS2) (28, 29, 67, 81). NO is also released following receptor-mediated stimulation of endothelial-type NOS, NOS3, or the neuronal isoform of the enzyme NOS1. With few exceptions, most cell types can be stimulated to express NOS2 (2, 48, 93, 94); however, the latter two NOS isoforms are constitutively expressed in specific cell types including smooth, cardiac, and striated muscle cells, neurons, endothelial and epithelial cells (2, 47, 52). Although the level and functional roles of NO production can vary between cell types, continuous release of high concentrations of NO is mediated by inducible NOS2, whereas the constitutively expressed isoforms NOS1 and NOS3 generally release lower levels of NO in a pulsate fashion (34, 48, 93, 94). In early studies, NO produced by the constitutively expressed NOS isoforms was viewed as a mediator in signaling cascades, whereas NO produced by inducible NOS2 was largely held responsible for nonspecific damage to microbes and mammalian cells (51, 95). This characterization of the same molecule as two-faced inevitably led to confusion about the nature of the physiological events resulting from NO production, and the overall beneficial or detrimental role of NO became a focus for debate (79). Current studies, which benefit from new information on the complex chemistry of this reactive free radical in diverse cellular microenvironments, have revealed that in cells, NO seldom diffuses throughout the cytosol; rather, this reactive mediator quickly interacts with available target molecules. Virtually every cellular component, proteins, lipids, sugars, and nucleic acids can be modified by NO modulating cellular activities and producing a host of altered molecules that are often differentially metabolized or compartmentalized. These modified molecules then act specifically to mediate diverse responses (31, 73, 117, 120, 132). A primary regulatory element in the actions of NOx is the proximity of target molecules to the NO source. Thus, it is now recognized that NO produced following induction of NOS2 may also act to transduce intracellular signals, and NO released from NOS1 or NOS3 may mediate cellular injury (11, 85, 132). The activities of the reactive radical gas are determined by the physiochemical nature of the

microenvirons where NO is released. Factors including the redox state of the cell, the availability of receptor molecules, the hydrophobicity of the local environment and the presence of other reactive mediators determine the proximal reactive NOx species and physiological events mediated by this molecular chameleon.

Cellular production of NO, via the activity of the NOSs, is regulated by a spectrum of interactions between cells and the extracellular environment. Cytokines, inflammatory mediators, and microbial products induce the expression of NOS2, and in some instances, hypoxia or cellular stress may also induce the enzyme (28, 29, 38, 122). The activity of NOS2 is continuous following expression of functional protein. Constitutively expressed NOS1 and NOS3 can be activated to produce NO through ligand–receptor interactions of neurotransmitters including acetylcholine, norepinephrine, *N*-methyl-D-aspartate (NMDA), and neurokinins, lipid mediators such as ceramides and platelet-activating factor, and following the activation of growth factor receptors by protein mediators such as vascular endothelial growth factor and endothelin (33, 86). It has also been reported that cellular stressors such as hypoxia, fluid shear stress, arrhythmias, and heat shock can up-regulate the expression and activity of the constitutive NOS isoforms (1, 4, 33).

PROTECTION, REPOSITORIES, OR STORES?

Within cells and tissues, interactions with other radical species comprise the most facile reactions of NO. In the presence of free $O_2^{\bullet}$ NO undergoes a free radical quenching reaction creating $ONOO^-$ at the rate of a diffusion-controlled reaction ($k = 1.9 \times 10^{10}\ M^{-1}\ s^{-1}$) (17, 90), a rate far in excess of the rate of enzymatic removal of this reactive oxygen radical (121). In a similar manner, reactions between NO and intermediates created during catalysis by active oxidases, such as the reaction of NO with bound lipid peroxyl radicals of 15-lipoxygenase, also proceed at rates that exceed those of antioxidant enzymes (41, 91, 125, 130). However, the most frequent cellular reactions involving NOx appear to be transient associations of these reactive mediators with cellular molecules. These reactions effectively sequester the gas, which can then be enzymatically or nonenzymatically released. Interaction with oxidants and reductants during the release process may influence the reactive nitrogen species released (78, 128). The reversible sequestering of NO plays a critical role in cell and tissue functioning. For example, in sepsis, activated immune cells release high levels of NO in the vasculature, which can act to damage cellular components. However, hemoglobin stores in red blood cells provide protection through transiently binding NO, thus effectively buffering the levels of free NOx (104). As a free radical, the interactions of NO with cellular oxidants and biomolecules are also fast, far exceeding rates of enzymatic catalysis. For example, similar to its interaction with oxygen intermediates, the reaction rate of NO with oxyhemoglobin has also been determined to be diffusion-limited; thus, oxyhemoglobin can act as a facile sequestering agent for free NO within the vascular lumen. In cells, the reaction of NO with iron-bound heme or other heme-containing proteins also transiently confines NO. In these instances, enzymes, such as the soluble guanylate cyclases, act as target molecules whose activities are specifically enhanced by binding NO (35, 93, 94, 99, 102). In other instances, such as reaction with catalase, cytochrome P450s, or ribonucleotide reductase, interaction with NO may inhibit enzymatic activity. At the same time, limiting rates of NO release from these enzymes functions to sequester the free radical gas efficiently (78, 99).

Other facile nonenzymatic reactions capable of effectively sequestering NO include reaction with metalloporphyrins, vitamin E, uric acid, β-carotene, and selenium compounds (73, 102, 134). The complex web of NO interactions with molecular oxygen, reactive oxygen species such as $O_2^{\bullet -}$, transition metals, lipids, nucleic acids, and proteins is far from completely elucidated. However, it is becoming increasingly apparent that many of these reactions may also lead to the release of NO and other reactive NOx from cellular stores resulting in both beneficial and detrimental effects (11, 73, 78). The concept that cell and tissues retain repositories of reactive NOx is an emerging one, and the

physiological significance is not yet fully defined. However, this resultant overall "nitrosative" state of cells must be viewed much in the same manner as the "oxidative" state when considering potential physiological effects of therapeutically modulating NO levels.

NO is a small molecule that swiftly transmutes to numerous other oxynitrogen species through an array of reactions (30, 42, 78). When addressing questions concerning the biologically relevant reactions of NO, it is important to consider the NOx most likely to mediate a reaction and the specific chemical and physical characteristics of these NOx that determine their compartmentalization. The enhanced solubility of NO in lipids over that in aqueous solutions combined with a rate of diffusion in lipid bilayers, which is estimated to be 1.4 times greater than the rate of oxygen diffusion in membranes (76, 98) indicates that in biological systems NO will preferentially partition to lipid compartments. Thus, NO may not be available for interaction with hydrophilic residues of cytosolic proteins, whereas the affinity for aqueous compartments is higher, and the migration through lipids is limited, for charged nitrogen oxides. This may result in a higher affinity of charged species such as $ONOO^-$ and nitronium (NO^+) for cytosolic rather than membranous molecular targets. Many of the contradictory conclusions drawn in earlier studies resulted from overlooking this significant factor.

BIOLOGICAL REGULATION BY PROTEIN NITROSATION

In the past decade, alteration of protein function by nitration has been established as an important mechanism for regulating cellular levels of NO. More recently, the importance of nitrosation for intracellular cellular signaling has been recognized (39, 73, 97, 110). However, protein nitration was viewed as a relatively minor component in the regulation of physiological function by NO. New insights into the complexity of NO chemistry in biological systems have dramatically altered this view (66, 98). Until recently, the reactive nitrogen oxide produced from L-arginine by NOS was believed to be exclusively NO. However, questions concerning the stoichiometry of NADPH consumption and the poorly defined role for tetrahydrobiopterin led investigators to devise reaction mechanisms that result in the direct formation of other NOx. In several elegant studies, both NO^- and $ONOO^-$, rather than NO itself, have been suggested as the reaction products produced by NOS. It is interesting to note that both NO^- and $ONOO^-$ can be metabolized to NO by the enzyme superoxide dismutase, potentially accounting for the appearance of NO in cells. *In vivo* $ONOO^-$, N_2O_3 [which is formed through the aerobic oxidation of NO to $^{\bullet}NO_2$ and its subsequent reaction with another molecule of $^{\bullet}NO$ (126, 127)], $^{\bullet}NO$–metal complexes, and NO^+ all preferentially act to nitrosate proteins. Although proteins can be nitrosated on available oxygens or nitrogens, *S*-nitrosation is generally the most facile and freely reversible reaction. *S*-Nitrosation is thought to mediate signaling, whereas *O*- and *N*-nitrosation and nitrations, which have limited reversibility, are believed to give rise to more permanent protein modifications that act primarily to trap NO (13, 66).

It has been speculated that the oxidation of the ferrous heme of hemoglobin by NO is an insignificant reaction *in vivo*, rather than that NO transiently nitrosates specific heme cysteine residues modulating vasodilatation. In this model, NO binds to Cys^{93} in oxygenated hemoglobin and is delivered to the microvasculature where, in a deoxygenated environment, the *S*-nitroso linkage is destabilized. NO molecules, free or complexed to small thiols, then diffuse through the erythrocytes to the vascular walls, causing vasodilatation of the local microvasculature and subsequent recruitment of additional erythrocytes (40). Although this has yet to be established *in vivo*, treatment of sickle cell anemia patients with inhalation of NO augmented NO transport to the microvasculature (56). Conversely, in other reports, treatment of human blood in vitro revealed that SNO-hemoglobin has a higher affinity for oxygen than native hemoglobin. This finding implies that NO transfer from deoxygenated SNO-hemoglobin *in vivo* would be limited to regions of extremely low oxygen tension (36). Interestingly, in recent studies it has been de-

termined that serum albumin may act as a sink for low-molecular-weight nitrosothiols, enabling the protein to act as a modulator of NO^+ transfer between the vascular wall and hemoglobin retained in red blood cells (36). Although the *in vivo* reactions between NOx and hemoglobin are still subject to debate, there are clear indications that an array of NOx–hemoglobin interactions modulate oxygen uptake and delivery.

Perhaps the best characterized covalent protein modification mediated by NO is the reversible *S*-nitrosation of protein cysteine residues. This has been established as a mechanism for physiological signaling regulating a variety of metabolic processes including apoptosis, muscle contraction, and gene expression. Alternatively, *N*- and *O*-nitrosation are associated with irreversible loss of protein function and pathophysiology rather than cell signaling. For example, $ONOO^-$ has been found to damage proteins through carbonyl formation on arginine and lysine residues, oxidation of methionine side chains, and nitration of both tyrosine and tryptophan residues. In numerous studies, the presence of long-lived nitrotyrosine residues on cellular proteins has been used as a marker for the presence of excessively high levels of NO (10, 114). Identification of nitrosated tyrosine residues is believed to indicate cellular injury mediated, in part, by NO (53, 82, 114). *In vivo*, *N*-nitrosation of glutathione reductase has been found to inactivate the enzyme (62). And a persistent danger of *N*-nitrosation is the promotion of mutagenesis and carcinogenesis from the nitrosative deamination of DNA bases via the formation of nitrosamine intermediates (65).

Other nitrosation reactions not currently considered significant in intracellular signaling but with the capacity to participate in these processes, as well as in sequestering free NOx, include reversible protein modification through sulfoxidation reactions leading to the creation of unstable sulfenates (RSOH). In subsequent reactions, as sulfenates decay, intramolecular disulfide bonds can be formed with available thiol residues, or mixed disulfides can be created through bonds formed with low-molecular-weight thiol compounds such as glutathione (GSH) (131). The modified proteins can then be readily restored to their original state through enzymatic or nonenzymatic reduction (118). Proteins activated by NO-mediated disulfide bond formation include heat shock protein 33, cRET encoded receptor tyrosine kinases, and the bacterial transcription regulatory systems oxy-R and sigmaR-RsrA (6, 56, 61, 101, 139). Similar oxidation reactions mediated by reactive oxygen species or NO can also regulate voltage-dependent potassium channels and calmodulin activity (20, 21). In addition, oxidation of methionine to methionine sulfoxides and sulfones has been found to inhibit DNA-binding activity of the high-mobility group protein in *Drosophila melanogaster* (27). Potentially, NO may act in a similar manner to regulate transcription in mammalian cells.

NITROSO-SIGNALING

It has long been recognized that the properties of *S*-nitroso proteins make them excellent candidates for signaling molecules (39, 73, 97, 110). Protein *S*-nitrosation is an enzymatically and nonenzymatically reversible process. *S*-Nitrosothiols are inherently unstable in aqueous solutions, and many *S*-nitroso proteins are short-lived in cells (73). In addition, interaction of NO with GSH and resultant *S*-glutathiolation and thiol oxidation provide another redox-sensitive pathway for protein regulation by NO (131). The emerging roles of these reactive species in intracellular signaling have been identified as important in various cellular processes including proteolytic processing, ubiquitination, and degradation of proteins, ion-channel activity, regulation of transcription, and tyrosine phosphorylation (73, 120, 133). Through these processes, NO has the capacity to be a facile regulator of an array of physiological responses including antioxidant and inflammatory responses, muscle function, cellular proliferation and differentiation.

One of the most striking findings confirming the physiological relevance of redox-regulated *S*-nitrosylation is the discovery that the activities of numerous cysteine proteases are regulated through this pathway. In early studies, it was observed that cytokine maturation and some apoptotic pathways are regulated by NO-

thiol *S*-nitrosation (RSNO). Current results indicate that seven members of the human caspase family of cysteine proteases are inactivated by *S*-nitrosation (109). The caspases are a family of cysteine proteases that specifically cleave protein after aspartate residues. The apoptotic mechanism inhibited in NO-sensitive apoptosis includes caspase-3 activation after down-regulation of Bcl-2 and up-regulation of Bax protein levels (71). Reversible *S*-nitrosation of caspase-3 inhibits its enzymatic function and interrupts the apoptotic process. During cytokine maturation, the secreted form of the proinflammatory cytokine interleukin 1 is released from activated macrophages and epithelial cells following cleavage of the apoprotein by caspase 1 [interleukin-1β-converting enzyme (ICE) protease (84)], this process is also actively regulated by *S*-nitrosation. In other studies, protein degradation and processing by cathepsin K, papain, calpain, and related cysteine proteases were reversibly inhibited through NO-mediated formation of mixed disulfide proteins (75, 107, 123, 124, 135). Most recently, the enteroviral protease coxsackieviral protease 2A that cleaves dystrophin, causing dilated cardiomyopathy, and the activity of human immunodeficiency virus-1 protease have been found to be regulated by *S*-nitrosation (7, 22). *S*-Nitrosation has also been found to inhibit the ubiquitin/proteasome pathway potentially through interfering with the activity of the ubiquitin conjugation system (37).

In gastric and other smooth muscles, the velocity of the calcium waves propagating action potentials is often too high to be mediated by simple diffusion. In these instances, local calcium-activated calcium release through ryanodine-sensitive channels acts to enhance wave velocity and more efficiently propagate the signal (14). The relative oxidant status of the cells has been reported to regulate the activation and inhibition of calcium release from these stores through differential *S*-nitrosation of ryanodine receptors. In the presence of physiological oxygen tensions, nanomolar NO activates the channels by *S*-nitrosating one specific cysteine residue. However, in cells exposed to high oxygen concentrations and/or higher NO levels, nitrosylation of multiple cysteine residues inhibits calcium release (32, 80, 137). Similar patterns of coupling calcium release from internal stores to voltage- and ligand-gated ion channels have been identified in airway smooth muscle, coronary arterial muscle cells, skeletal muscle, cardiac muscle, human myometrium, corpus cavernosum, and mouse parotid acini (16, 24, 44, 58, 83, 129). In other NOx-modulated electrochemical processes, stimulation of NMDA receptors in excitatory neurons can be associated with toxicity. Among the events that may initiate this process are the pathological conditions resulting from hyperhomocysteinemia. It has been discovered that *S*-nitrosation of critical NMDA receptor cysteine residues produces a protective blockade and ameliorates homocysteine-induced toxicity (25, 69, 70). In additional studies, *S*-nitrosation has been found to be important in the regulation of sympathetic neurotransmission through inhibition of the uptake of norepinephrine, while having no effect on dopamine transport (63, 64).

Although the role played by NO in long-term potentiation is still poorly defined, recent reports indicate that this process may be modulated through *S*-nitrosation of neurogranin. This protein accumulates in the dendritic spines and shafts of neurons located in the cerebral cortex, hippocampus, and striatum of the adult brain and has been proposed as a postsynaptic second messenger in signaling regulating long-term potentiation and depression. *S*-Nitrosation weakens the binding affinity of neurogranin for calmodulin and frees the calmodulin for other calmodulin-dependent enzymes, facilitating the induction and maintenance of long-term potentiation (50). Nitrosation and nitration have also been found to alter the activities of other protein kinases and kinase substrates. Nitration induced dephosphorylation of two mitogen-activated protein (MAP) kinases p38 and extracellular signal-regulated kinase ERK, and was found to reduce the activity of these enzymes in murine kidney cells. In addition, increased phosphorylation of c-Jun NH_2-terminal kinase/stress-activated protein kinase JNK in response to nitrosation was observed, suggesting activation of this signaling pathway by nitrogen oxide-mediated

stress signals (72). In vascular smooth muscle cells, nitrosation enhances platelet-derived growth factor induction of protein kinase Bα (108) and inhibits cellular proliferation associated with up-regulation of p21 potentially through a p42/44 MAP kinase signaling cascade (54, 68).

Regulation of transcription through nitrogen oxide-mediated regulation of eucaryotic transcription factors may be important in cellular adaptation to redox-related stress. Recent studies have determined that protein nitration can result in increased levels of active c-fos and c-jun in lung epithelial cells (58, 89). Regulation of DNA binding activity by nitrosation has been demonstrated for several redox-sensitive transcription factors including nuclear factor-κB, activator protein-1, and p53 (89). Factors regulating intracellular oxidant levels, which are induced by nitrosative events, include heme oxygenase-1, the rate-limiting enzyme in the degradation of heme (15), and the human platelet L-arginine transporter (49).

Mixed disulfide formation of *S*-nitrosated proteins interacting with the ubiquitous cellular reducing agent GSH has recently been investigated as a potential mechanism in cellular adaptation to oxidative stress (5, 46). In this regard, the transient accumulation of mixed disulfides in response to oxidants or radical-generating ultraviolet light or during inflammation has been well characterized (3, 26, 46, 87). Additionally, cellular processes that are reversibly modulated following oxidant-mediated *S*-glutathiolation have been identified. Several diverse metabolic processes regulated in this manner have been identified including growth and differentiation mediated by tyrosine phosphorylation, ubiquitination, transcription, energy metabolism, and fatty acid synthesis (8, 9, 12, 23, 43, 55, 106, 116, 119). More recently, NO-mediated *S*-glutathiolation has been found to play a role in aging (112, 126, 127), bone and cartilage metabolism (62, 88, 107), and cellular antioxidant defense (49, 59, 74).

The complex chemistry of NO–GSH interactions under aerobic conditions has been compared to the reaction of glutathionyl radicals with oxygen (55, 77, 111, 116). It has been postulated that in the oxygenated intracellular environment $GS^{\bullet}$ can undergo both quenching chain-perpetuating reactions with itself or GS^{-} forming $^{\bullet}GSSG^{-}$. Alternatively, $GSOO^{\bullet}$ formed following interaction of $GS^{\bullet}$ with oxygen may interact with another molecule of GSH forming unstable sulfenyl hydroperoxide (GSOOH) and $GS^{\bullet}$. Reaction with GSH transiently forms sulfenic acid that, as described above, quickly interacts with protein thiols to form intermolecular disulfide bonds or GSH forming GSSG.

It is important to consider that many of the cellular proteins reversibly *S*-thiolated during oxidative stress including actin, lens crystallins, glutathione-*S*-transferase, glyceraldehyde-3-phosphate dehydrogenase, creatine kinase, superoxide dismutase, and carbonic anhydrase can also undergo NO-induced thiolation (19, 73, 100, 103). These reactions provide an important link between cellular oxidative and nitrosative status, as well as potentially mediating the sequestering-release processes required for maintaining cellular stores of NO.

Emerging evidence provides support for the hypothesis that reversible interaction of reactive NOx with cellular components provides a mechanism for dynamic regulation of cellular function. In addition, it is anticipated that ongoing studies concerning the regulation of protein functioning by NOx-mediated posttranslational modifications will provide information on the physiological and pathological processes regulated by these facile reactions. In addition, appreciation of the presence of a potential intracellular pool of NOx is likely to provide important insights into the complex pathologies of multifactorial disorders including septic complex shock, cardiac myopathy, and neurodegeneration.

ABBREVIATIONS

GSH, glutathione; MAP, mitogen-activated protein; NMDA, *N*-methyl-D-aspartate; $NO^{\bullet}$, nitric oxide; NO^{+}, nitronium; $^{\bullet}NOO$, $^{\bullet}NO_2$, nitrogen dioxide radical; NOS, nitric oxide synthase; NOS1, NOS2, and NOS3, the neuronal, inducible, and endothelial isoforms of NOS, respectively; NOx, nitrogen oxides; $O_2^{\bullet -}$, superoxide; $ONOO^{-}$, peroxynitrite; RSNO, nitroso.

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