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Cardiovascular Research 62 (2004) 43–52

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

S-nitrosylation: a potential new paradigm in signal transduction

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Received 24 November 2003; received in revised form 26 December 2003; accepted 8 January 2004

Time for primary review 23 days

Abstract

Much attention has been paid to nitric oxide (NO) research since its discovery as a physiological mediator in the cardiovascular system. In recent years, newer roles have been attributed to this molecule and its close relatives, termed collectively reactive nitrogen species (RNS). These roles relate to different mechanisms of protein modification, among which S-nitrosylation of cysteines has emerged as a potential new paradigm in signal transduction and regulation of protein function. We review here the chemical basis of this modification compared with other protein modifications related to nitric oxide, as well as the kind of specificity we can expect from it. We also review the current methodologies that can be applied to the study of S-nitrosylation and identification of S-nitrosylated proteins in cells, and detail the relevance of this modification in several proteins related to cardiovascular system.

Keywords: Signal transduction; Nitric oxide

1. Introduction

Since the discovery of nitric oxide (NO) as a physiological mediator in the early 1980s, research devoted to the biological role of this molecule has evolved spectacularly, both in its intensity and extent. While the roles of NO as a modulator of vascular tone, neurotransmission and an effector in some immune responses are widely recognized, newer and attractive roles have emerged over the past 10 years. These relate to the capacity of NO to interact with and modify a wide variety of other molecules: free radicals such as the superoxide anion, key redox regulators such as glutathione, and macromolecules (DNA and proteins). This lends a basis for NO to regulate crucial processes within the cell such as the response to redox perturbations, protein function, and gene expression through non-enzymatic modifications. S-nitrosylation of proteins is one potential mechanism underlying these events that has attracted increasing interest in recent years. In the first part of this review, we briefly describe the chemical basis of the interaction of NO with heme groups, thiol groups and other radicals before discussing the specificity of these processes and describe the spatio-temporal conditions which might lead to S-nitrosylation within cells. In the subsequent sections, the currently available methodologies for the study of S-nitrosylation are reviewed, and examples of S-nitrosylation are examined in detail, with special focus on characterized in vivo examples and on proteins related to the cardiovascular system.

2. The chemical basis of NO–protein interactions

Nitric oxide can modify proteins via different chemical processes. These either involve direct interaction with the protein by NO itself, or the prior production of "reactive nitrogen species" (RNS), formed as a result of the interaction of NO with other radicals and oxygen. The most well-known direct modification is the formation of complex (or coordinating) bonds between NO and transition metal ions within heme groups, this being the basis for the activation of soluble guanylate cyclase [1]. Other examples of NO interactions with heme groups include cytochrome P450

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0008-6363/$ - see front matter © 2004 European Society of Cardiology. Published by Elsevier B.V.
doi:10.1016/j.cardiores.2004.01.013

[2] and haemoglobin [3]. NO can also bind to non-heme iron proteins [4] and other transition metals. In the case of cytochrome *c* oxidase (complex IV of the mitochondrial respiratory chain), which contains two hemes and two copper centres, NO and oxygen compete for the binding to the active centres, but the exact mechanism of enzyme inhibition by NO is still under discussion [5,6].

Incorporation of NO moieties by covalent bonding to protein groups is chemically possible in the case of cysteine thiols, tryptophan indols and amines (lysine and N-terminal) [7–9], although S-nitrosylation, the formation of the S–NO bond by cysteine thiol nitrosylation, can be considered the most important, because of its higher reactivity, occurrence in physiological conditions and its influence on many protein functions.

The usage of the terms "nitrosation" and "nitrosylation" needs some clarification. From the chemical point of view, "nitrosation" means the addition of a nitroso group, i.e., the NO diatomic group; "nitrosylation" means the addition of a nitrosyl group, NO, stressing the concept of the addition of a chemical group that, if it were free, it would be a radical (in analogy to other chemical additions containing the -yl- particle: acetylation, phosphorylation, etc.). The fact is that in the nitric oxide species, both atomic groups are the same, as the radical is in itself relatively stable. Even so, some authors prefer to distinguish the incorporation of the ·NO radical to a metal by a complex (or coordinating) bond as "nitrosylation", and the covalent incorporation of a NO diatomic group to another chemical group (regardless of the reaction mechanism) as "nitrosation" (in accordance to the nomenclature, which uses the term "nitroso" when defining the names of the resulting compounds). However, the increasing recognition of the functional importance of this post-translational modification, and the widespread inclusion of the particle "-yl-" in terms describing other post-translational modifications (glycosylation, phosphorylation) has led pioneer investigators in the field to make a case for the use of nitrosylation both for thiols and metals [10,11]. In any case, incorporation to a thiol can be clearly distinguished because of the prefix "S-", referring to the incorporation of the NO moiety to a sulfur atom to form the S–NO bond: "S-nitrosation" or "S-nitrosylation".

It is also important to distinguish nitrosylation/nitrosation from nitration, a term which describes incorporation of a nitro triatomic group ($-NO_2$) and which, in protein chemistry, is generally used to describe the incorporation of that group at position 3 of the phenolic ring of tyrosine residues. This modification has also been thoroughly studied in recent times, an is clearly related to the formation of peroxynitrite (for reviews, see Refs. [12,13]).

It is generally accepted that direct reaction of the ·NO radical with a thiol does not yield the nitrosothiol [14,15], although a mechanism with additional participation of oxygen species has been proposed [16]. Previous reaction of NO with O_2 is considered to be necessary for S-nitrosylation, via formation of higher nitrogen oxides, among which N_2O_3 is thought to be the quintessential S-nitrosylating species [17,18]. This reaction of NO and O_2 can be favoured in membrane environments, where NO and O_2 may reach higher concentrations [19], or even in hydrophobic protein microenvironments [20]. N_2O_3 can be partially dissociated into $[^{+}ON\cdot\cdot NO_2^{-}]$, which favours the reaction of the nitrosonium (NO^{+}) moiety with the nucleophile sulfur atom. Thus, S-nitrosylation can be better understood considering the transfer of nitrosonium and not of ·NO. This happens also in the process of transnitrosation, or transfer of the S-nitrosylation between a nitrosothiol and another thiol [21–23]. This mechanism of protein S-nitrosylation is of biological relevance, especially due to the high concentrations of intracellular low molecular mass thiols like glutathione. Nitrite is also a nitrosylating agent in acidic environments, where it forms nitrous acid, HNO_2 [11,24], although its occurrence in physiological systems would be restricted to highly acidic environments.

Formation of nitrosothiols can be favoured in more ionisable cysteines, such as those in which the thiolate anion can be stabilized by acid–base interactions with neighbouring groups, either belonging to adjacent residues in the primary sequence (which is the basis of the consensus sequence proposed by Lipton and Stamler [25]) or just in the proximity in the three-dimensional structure [26–28]. Thus, in the context of such an "acid–base motif", the pK_a of different cysteines in a protein would be an important factor in determining the occurrence of S-nitrosylation. It is important to note that in many instances where S-nitrosylation has been described, the protein cysteines involved are also oxidized, and tend to form disulfide bonds. Also, nitrosylation of thiols can chemically induce the formation of a disulfide bond [21]. Given that the most abundant thiol in the cytoplasm is glutathione, S-nitrosylation is likely to promote S-glutathionylation: the incorporation of glutathione into proteins via mixed disulfide bonds. S-glutathionylation is increasingly recognized as an important post-translational modification capable of regulating protein function [29–31]. This kind of reactive environment for cysteines is commonly encountered in the active centres of enzymes, and might favour the alteration of protein function by S-nitrosylation (or by other thiol modifications).

3. Specificity and spatio-temporal conditions for S-nitrosylation

Many comparisons have been made between S-nitrosylation and phosphorylation as signalling mechanisms in the cell [32,33]. The important issue here is not whether S-nitrosylation is used by the cell as a signalling mechanism, but rather to understand the particular ways in which the cell can use it, and try to delimitate its specificity and cellular functionality.

Although both S-nitrosylation and phosphorylation are covalent modifications of protein residues, the crucial dif-

ference is that phosphorylation is enzyme driven, whereas S-nitrosylation is achieved through the non-catalysed chemical modification of a protein residue. Thus, in S-nitrosylation, the reaction specificity does not rely on the recognition of a target structure by an enzyme. Instead it depends solely on the chemical reactivity between the nitrosylating agent and the target. Several factors can be identified as involved in this kind of specificity.

3.1. Reactivity of the target protein residue

Analysis of S-nitrosylation in several proteins shows that not all protein cysteines that remain in the free-thiol state become nitrosylated [27,34,35]. Finding a common structural (sequence or three-dimensional) motif that might determine this reactivity has not been straightforward. As we have already seen, more ionisable cysteines are among the more nitrosylable, but cysteines in hydrophobic environments may also be candidates for S-nitrosylation. Thus, as the residue environment is what determines its reactivity, chemical specificity does not occur at the molecular (protein) level, but at the "submolecular" (atomic) level. Also, as the modification can affect similar groups in different proteins, the concept of "plurimolecular" functional specifity has been postulated [36].

3.2. Concentration

While chemical reactivity can be expressed in terms of thermodynamic and kinetic constants, the occurrence of a reaction is determined also by the concentrations of the species involved: in this case, the nitrosylating agent and the protein. This basic chemical concept is usually forgotten when biochemical pathways are described because the enzymatic catalysis diminishes its influence. In the case of S-nitrosylation, where the rate of the chemical reaction is not enzymatically determined, this point can be crucial. It depends on the following facts:

(1) *Production of NO and related RNS*. NO production is a key step in S-nitrosylation, and originates by a tightly regulated group of enzymes, the nitric oxide synthases (NOS). As NO is not the main nitrosylating agent, formation of other RNS, for example by reaction with O_2 or O_2^-, has to be considered, taking into account that the NOS enzymes can also be responsible for the production of different RNS and ROS, including O_2^- [37,38] (Fig. 1).

(2) *Localization*. Subcellular compartmentalization can be extremely important in determining local concentrations of biochemical reagents. There is growing evidence that at least some S-nitrosylable proteins are physically associated to eNOS or nNOS (reviewed in Refs. [33,39–41]), and that the subcellular compartmentalization of these enzymes is tightly regulated. Using a modified yeast two-hybrid system, a recent report screened protein interactions of procaspase-3 (a known target of S-nitrosylation) and confirmed that this protein interacts with eNOS, nNOS and acid sphingomyelinase inside cells [42]. Localization specificity may not apply to iNOS, as the amounts of NO generated by iNOS are of a higher magnitude, and form part of a non-

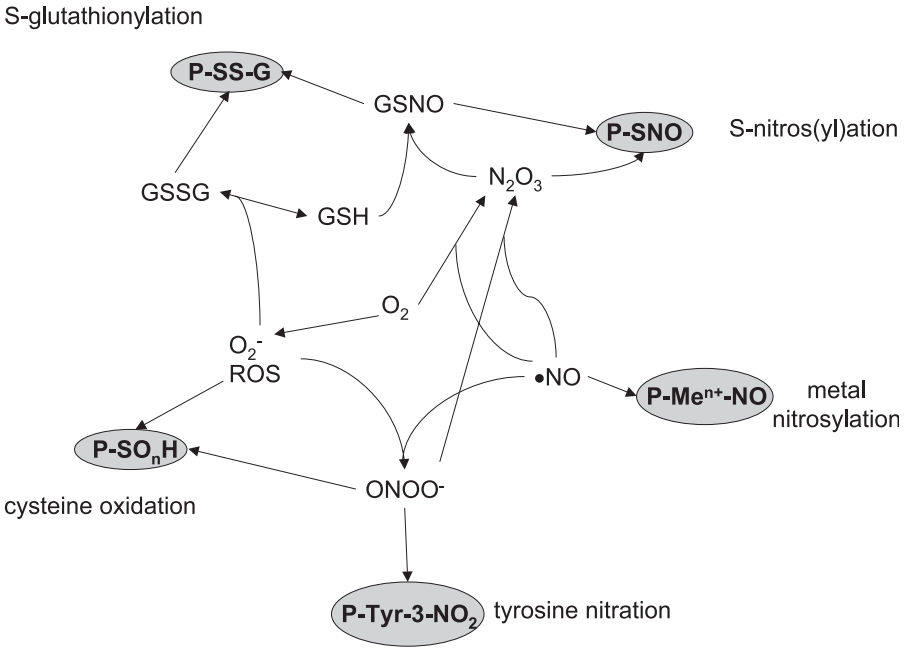

Fig. 1. Chemical relationship among different reactive oxygen species (ROS) and reactive nitrogen species (RNS), and their impact on some post-translational modification of proteins. GSH: glutathione; GSSG: glutathione disulfide; GSNO: S-nitrosoglutathione. ROS chemistry is simplified in the scheme by O_2^- formation from O_2. Arrowheads which join mean reaction among species.

constitutive response programme related to infection and inflammation [43].

3.3. Stability of the bond

S-nitrosylation is a very labile covalent modification under physiological conditions. As with its synthesis, cleavage of this bond can occur without the help of specific enzymes, although several enzymes have been described which can break at least low mass S-nitrosothiols at a relatively high K_M (reviewed in Ref. [39]). The cleavage reaction might be accelerated by reaction with transition metals [44], especially copper(I) [24], or by transnitrosation, among other mechanisms. Some researchers have stressed the potential of denitrosylation as an important intracellular signalling mechanism in its own right, and this area requires further investigation [45,46].

4. Current methodologies for the detection of S-nitrosylation

The major methodological problems in the study of protein S-nitrosylation are the lability of the S–NO bond and the fact that the level of endogenously nitrosylated proteins in the cell can be very low, hampering in vivo detection of individual S-nitrosylated proteins (Fig. 2). Thus, although over 100 proteins have been reported to be S-nitrosylated in vitro, very few have been described in their cellular environment [32]. Moreover, artifactual nitrosylation can be produced during manipulation, so S-nitrosylation of a protein should be confirmed by several methods.

There are a number of methods for the "direct" detection of the S–NO bond. These are measurement of its characteristic absorbance band at 340 nm (although the sensitivity of this method is low); electrospray ionization mass spectrometry (ESI-MS) [47–49] (the widely used laser ionization, MALDI, breaks the S–NO bond [50]); electrochemical detection after liquid chromatography [51]; and NMR with ^{15}N [52]. "Indirect" chemical methods require cleavage of the S–NO bond and detection of the species formed, NO or nitrite. Ozone chemiluminescence can measure NO released from nitrosothiols by photolytic cleavage [51], specific reduction with Cu^+/cysteine at pH 6 [53], or reduction with I_2/I^- [54]. Heterolytic cleavage with $HgCl_2$ produces nitrite, which can be measured by colorimetric or fluorometric methods [55,56].

The methods outlined above all provide a measure of the amount of nitrosothiols in a sample. To detect individual S-nitrosylated proteins in cells, prior immunoprecipitation with specific antibodies followed by reduction with Cu^+/cysteine or I_2/I^- and chemiluminescence has been used [57,58]. The sensitivity of this approach is, however, limited by the amount of protein immunoprecipitated. A rabbit antiserum raised against S-nitroso-BSA is commercially available (Calbiochem) and can be used in some immunological applications. Its specifity can be verified by comparison with a sample treated with $HgCl_2$ in which the nitrosothiols are broken [46]. Successful immunoprecipitation with this antibody followed by detection of individual proteins by immunoblot has been described only recently [59]. The "biotin switch" method

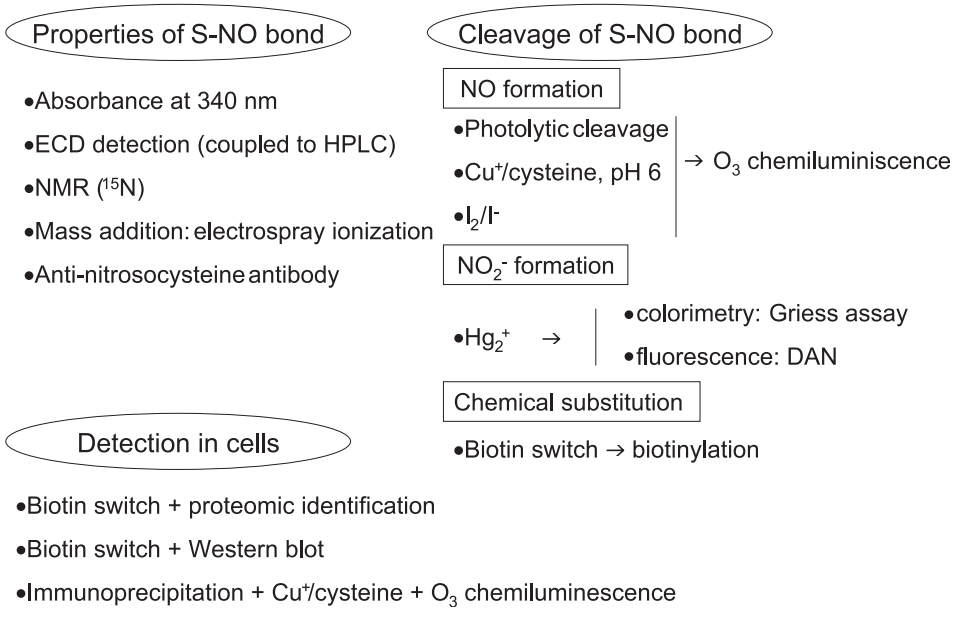

Fig. 2. Overview of techniques for detection and quantification of S-nitrosylated proteins. See text for details and references. ECD: electrochemical detection; NMR: nuclear magnetic resonance.

(see below) has also been used in combination with immunoblot to confirm modification of individual proteins [60].

The rapid development of proteomics in recent years has made possible the study of the proteomes, the pools of proteins present in cells, under different conditions. A major goal of specialized proteomics is the characterization of subproteomes that undergo specific post-translational modifications. Regarding S-nitrosylation, the study of the "nitrosylome" (that pool of proteins which are S-nitrosylated in cells) would identify new targets for this modification. More specifically, this approach could be used to evaluate the feasibility of the signal transduction mechanisms postulated on the basis of in vitro studies. Again, technical difficulties have hampered study in this area, and the only approach which has so far been fruitful is an indirect one, called the "biotin switch" method, in which the cysteine-bound NO is replaced by a biotin label [60]. Several chemical steps are required for this conversion, and the method is designed to be sufficiently specific [60], although it lacks sensitivity [33]. Proteomic identification of S-nitrosylated proteins with the biotin switch method has been achieved in extracts from brain [60] and mesangial cells [61], and we have applied the method to intact endothelial cells [62]. In all cases, extracts or intact cells were first treated with nitrosylating agents, thus leading to elevated levels of S-nitrosylated proteins. Further efforts will be needed to increase the sensitivity of proteomic methodologies so that they can be applied in more physiological conditions, where S-nitrosylation might be more subtly regulated.

5. Regulation of protein function by S-nitrosylation

S-nitrosylation has been shown to modify the function of many proteins in vitro (for a list, see Ref. [63]). In the following section, we describe examples where the phenomenon has been well characterized, and which are all related to the common themes of gene expression regulation or relevance for the cardiovascular system.

5.1. $P21^{Ras}$

The oncoprotein GTPase $p21^{Ras}$ is one of the best-characterized examples of S-nitrosylation of a protein, and of how this might affect its signalling properties. First, it was described that NO and higher nitrogen oxides (NO_x), activated $p21^{Ras}$ signalling in cells, and that this protein was implicated in the activation of NF-κB by NO. This study with recombinant $p21^{Ras}$ showed that this effect was associated with the formation of 1 nitrosothiol per molecule [34]. The modification was confirmed by ESI-MS [47], and the affected residue mapped to cysteine 118 [48], which is located in a highly conserved motif (NKXD) implicated in nucleotide binding. Detailed structural studies using NMR have shown that the protein forms that are S-nitrosylated or mutated in the cysteine do not differ from unmodified $p21^{Ras}$ in their overall structures. The activity of the mutant protein is normal, although it is not influenced by nitric oxide [28,64]. Recent work has established that stably nitrosylated p21Ras does not differ in activity from the unmodified form, and the activity is altered only during the process of the nitrosylating reaction itself [28]. Thus, further information on the actual role of S-nitrosylated Ras is needed.

5.2. NF-κB

This transcription factor is involved in the response to many different stimuli, including oxidative stress, and regulates the expression of a wide variety of genes. These include interleukin 2, NOS2 (iNOS gene), and tumour necrosis factor α, all of which participate in crucial biological pathways such as inflammation and the protection from apoptosis. It has a complex regulation, its release from the inhibitory molecule IκB being a key step. This protein is degraded in a highly regulated way, which allows NF-κB to translocate to the nucleus where it can bind to DNA and activate transcription [65]. Although it is activated in situations of oxidative stress, the binding of NF-κB to *cis* regulatory elements depends on a specific cysteine in the p50 subunit, cysteine 62, being in the reduced state [66,67]. This cysteine is located in the DNA-binding region, and is surrounded by basic residues which confer a high reactivity to it. It is susceptible to oxidation and formation of disulfide bonds, both with another p50 molecule and with glutathione [66,68].

A role for p50 cysteine 62 has been established in the inhibition by S-nitrosothiols of recombinant NF-κB binding to DNA [69]. De la Torre et al. [70,71] confirmed S-nitrosylation of recombinant p50, and calculated its DNA binding kinetics. However, NO can influence the intracellular activity of NF-κB in many ways (reviewed in Refs. [72]), and against this background the importance of p50 S-nitrosylation remains unproven. Only two reports exist confirming the presence of this modification inside cells. Both studies relied on the immunoprecipitation of overexpressed p50 from cells, with detection of the nitrosothiol by photolysis-chemiluminescence in the case of kidney cells [73], and absorbance at 340 nm in macrophages activated by LPS [74]. There are also reports of inactivation of DNA-binding activity consistent with S-nitrosylation, but the link has not been demonstrated definitively [75,76]. Interestingly, S-nitrosylation of p50 may be dependent on the cell type [76].

5.3. Hypoxia-inducible factors (HIF)

Hypoxia-inducible factors (HIF) are a family of transcription factors that are activated in response to low oxygen concentrations, and they form part of the PAS

superfamily of conditional transcription factors [77]. They are heterodimers of two PAS proteins: a constitutive subunit, called ARNT ("aryl receptor nuclear translocator") or HIF-β; and a regulatory one called HIF-α. In humans, two α subunits have been described: HIF-1α and HIF-2α, also known as EPAS ("endothelial PAS protein"). Under normoxic conditions, these subunits are specifically hydroxylated at proline and arginine residues, and these modifications promote ubiquitination and degradation, as well as inhibiting the transactivation capacity. Under hypoxic conditions, these hydroxylations are impaired, leading to accumulation of HIF, translocation to the nucleus and restoration of the transactivation capacity. When activated, HIFs induce a number of genes, such as erythropoietin or VEGF, involved in responses to hypoxia.

The effect of NO-related compounds on HIF activation is complex, and is currently an area of active research. A dual role for NO, depending on the cellular O_2 concentration, is now clear. This explains initial observations that NO (as well as CO) inhibited hypoxic activation of HIF [78,79] while, under normoxic conditions, NO activates HIF activity in different cell lines [80,81]. The latter mechanism has been explained by NO-mediated inhibition of the Fe^{2+}-dependent activity of the prolyl hydroxylases, which leads to accumulation of the HIF-α subunits [82,83]. In this scenario, NO is acting as an autocrine or paracrine mimicker of hypoxia. The dual effect considered in this way has been thoroughly reviewed recently by Brüne [84]. More recently, it has been found that, during 1% hypoxia, the previously observed competitive inhibition of mitochondrial respiration by added NO increases the availability of O_2 in the cell. Moreover, the effect of NO in HIF-1α stabilization could be mimicked by mitochondrial respiration inhibitors in a ROS-independent manner. This suggests that the availability of O_2 for other enzymatic systems in the cells would be increased by NO's repression of mitochondrial respiration, increasing the activity of the prolyl hydroxylases [85].

Recent observations suggest that the effect of endogenously produced NO depends on its concentration, and published data in the literature should be interpreted in light of this. When NO is produced at high concentration (>1 μM), it promotes stabilization of HIF-1α, both in normoxia and relative hypoxia (3%), probably by inhibiting the prolyl hydroxylases. However, when NO is produced at low concentrations (<400 nM), its effect is only seen in hypoxia, when it impairs HIF-1α in a mitochondria-dependent manner [86]. This is thus an example of how the availability of molecules like O_2 and NO, which can be thought of as chemical mediators or effectors, can be critical for the perception of cellular signals that they affect.

HIF is also subject to regulation by more subtle mechanisms, which can might explain some of the observed effects of NO. Transactivation activity is enhanced by binding to CBP/p300. This binding is also controlled by hydroxylation, in this case of an asparagine residue [87], and recent reports revealed that the S-nitrosylation of cysteine 800 of HIF-1α favours this interaction [88,89]. However, these studies rely on the indirect "biotin switch" method of detecting S-nitrosylation, which is not reliable for the quantification described. Finally, there might be a divergence in the regulation of the two known human HIF-α subunits. EPAS/HIF-2α bears a cysteine in the DNA binding domain which is substituted by serine in HIF-1α, and which is subject to redox regulation [90], probably including S-nitrosylation.

5.4. Zinc finger transcription factors

The majority of transcription factors include zinc fingers as DNA binding motifs. These are folding motifs that contain a zinc atom bound to four complexing residues, cysteines and histidines, with the cysteines being in the thiolate form. Research published during the last years suggests that these structural motifs that bind DNA could also act as sensors of oxidative or nitrosative stress, triggering integrated programmes of gene regulation by structurally related transcription factors (reviewed in Ref. [91]). S-nitrosothiols can inhibit the DNA binding activity of the transcription factors Sp1 and EGR-1, and of nuclear receptor superfamily members such as the vitamin D_3 receptor (VDR), the retinoid X receptor (RXR) and the glucocorticoid receptor [92–94]. This inactivation might result from S-nitrosylation of a thiolate group involved in zinc binding. This would disrupt the structure of the zinc finger, and perhaps lead to other cysteine modifications, such as disulfide formation. It is interesting that these zinc fingers are protected from modification when they are bound to DNA, and the functionality of zinc fingers was restored by treatment with dithiothreitol [92,93], which could control the signal response.

One possibility is that an increment of nitrosylating reagents inside cells would reduce transcription driven by zinc finger transcription factors. This has been demonstrated in the case of IL-2 expression in response to IL-1β, which is dependent on Sp1 and EGR-1, and which can be inhibited by S-nitroso-L-cysteine [92]. This phenomenon is seen in reverse in the case of TNF-α expression, where Sp1 acts as a repressor. In this case, its inactivation by NO leads to an enhanced expression of TNF-α [95].

It is important to consider the general availability of Zn^{2+}, as this is also affected by nitrosylating agents. The main Zn^{2+} reservoir, metallothionein, accumulates Zn^{2+} by complexing it to cysteine centres, and releases it in response to Zn^{2+} requirements [96]. S-nitrosylation of metallothionein thiolates releases Zn^{2+} [97–99]. Thus, importance should be given to the relative sensitivities of different protein thiolates to S-nitrosylation and Zn^{2+} binding, because the function of Zn^{2+} binding domains could be altered in different ways in situations where the nitrosylating reagents are limiting.

5.5. Matrix metalloproteinases (MMP)

Matrix metalloproteinases are endopeptidases that are able to degrade extracellular matrix components. In the vascular wall, they are implicated in many physiological and pathological processes that require matrix remodelling, such as arteriosclerosis, angiogenesis, formation of aneurysms, wound healing, and tumour invasion. Acting through cGMP-dependent activation of signalling cascades, NO may have opposite effects on the expression of different MMPs. For example, induction has been reported for MMP-13 in endothelial cells [100,101], whereas abrogation of MMP-9 was reported in smooth muscle cells [102–104].

Direct activation of MMP-9 activity by S-nitrosylation of the so-called "cysteine switch" has been reported [105]. The catalytic activity of MMPs relies on a Zn^{2+} atom coordinated to three histidine residues in the MMP catalytic domain. MMPs are secreted in an inactive form as proproteins. These contain a sequence, common to many family members, which includes a cysteine that coordinates to the catalytic Zn^{2+}(the "cysteine switch"), rendering it inactive. Generally, activation of the protein is achieved by proteolytic cleavage of the propeptide by another molecule of the same or a different MMP. S-nitrosylation of the cysteine switch in MMP-9 allows it to dissociate from the metal ion and be active in the uncleaved form [105]. Since other MMPs share the same inhibitory motif, the possibility exists that this might be a general mechanism among MMPs.

5.6. Ryanodine receptor

The ryanodine receptor is a Ca^{2+} channel that is highly expressed in the sarcoplasmic reticulum of skeletal and cardiac muscle. Initial investigations into the influence of NO were contradictory, with reports showing that NO could both inhibit or activate the channel in both muscle types [106–108]. The first description of S-nitrosylation of the channel reported that many cysteines are S-nitrosylated (there are about 100 cysteines in each monomer of the functional tetramer, with half of them remaining in the free thiol state), and that this affects the channel activity [109]. Other researchers found that the effect of NO varies depending on several parameters, including the type of NO donor used and even when different S-nitrosothiols were employed [110,111]. This was supported by subsequent findings that different thiols in the protein react in different ways, but that specific nitrosylation occurs at just one cysteine, under the influence of the O_2 concentration [35,112–114]. Recently, the different reactivity of the thiols has been also highlighted when it was shown that S-nitrosoglutathione can exert different functions by promoting S-glutathionylation of cysteine residues different from the one that is S-nitrosylated [115].

6. Conclusions and perspectives

S-nitrosylation is a biochemical modification that potentially plays an important, and conceptually novel, regulatory role in signal transduction. The increasing evidence of functional changes resulting from this modification, and the growing number of proteins shown to be S-nitrosylated in vitro support this contention, and confirm this as an attractive area of research. Detailed studies addressing S-nitrosylation in vivo by endogenous NO and RNS are not abundant, but those that are available pave the way for future developments. Complementary approaches combining proteomic studies with the physiological generation of NO, together with studies to unravel the routes of enzymatic regulation will become increasingly feasible as improved methodologies enter the field. Coming years are likely to see the establishment of S-nitrosylation as a new paradigm in the regulation of protein function.

Acknowledgements

Research in our laboratory is supported by grants from the Plan Nacional de I+D+I, Ministerio de Ciencia y Tecnología (SAF 2000-0149 and SAF 2003-01039), Comunidad Autónoma de Madrid (08.4/0030.1/2003) and Ministerio de Sanidad (RECAVA network), all in Spain. A.M.-R. has received fellowships from the Fundación Ferrer Investigación and the Comunidad Autónoma de Madrid (co-financed by the European Social Fund).

References

[1] Stone JR, Marletta MA. Soluble guanylate cyclase from bovine lung: activation with nitric oxide and carbon monoxide and spectral characterization of the ferrous and ferric states. Biochemistry 1994; 33:5636–40.
[2] Wink DA, Osawa Y, Darbyshire JF, et al. Inhibition of cytochromes P450 by nitric oxide and a nitric oxide-releasing agent. Arch Biochem Biophys 1993;300:115–23.
[3] Gow AJ, Stamler JS. Reactions between nitric oxide and haemoglobin under physiological conditions. Nature 1998;391:169–73.
[4] Cooper CE. Nitric oxide and iron proteins. Biochim Biophys Acta 1999;1411:290–309.
[5] Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome *c* oxidase. Biochim Biophys Acta 2001; 1504:46–57.
[6] Moncada S, Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? Nat Rev Mol Cell Biol 2002;3:214–20.
[7] Simon DI, Mullins ME, Jia L, et al. Polynitrosylated proteins: characterization, bioactivity, and functional consequences. Proc Natl Acad Sci U S A 1996;93:4736–41.
[8] Zhang Y-Y, Xu A-M, Nomen M, et al. Nitrosation of tryptophan residue(s) in serum albumin and model dipeptides. Biochemical characterization and bioactivity. J Biol Chem 1996;271:14271–9.
[9] Espey MG, Miranda KM, Thomas DD, et al. A chemical perspective on the interplay between NO, reactive oxygen species, and reactive nitrogen oxide species. Ann NY Acad Sci 2002;962:195–206.

[10] Myers PR, Minor Jr RL, Guerra Jr R, Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. Nature 1990; 345:161–3.
[11] Stamler JS, Simon DI, Osborne JA, et al. S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds. Proc Natl Acad Sci U S A 1992;89:444–8.
[12] Turko IV, Murad F. Protein nitration in cardiovascular diseases. Pharmacol Rev 2002;54:619–34.
[13] Ischiropoulos H. Biological selectivity and functional aspects of protein tyrosine nitration. Biochem Biophys Res Commun 2003; 305:776–83.
[14] Pryor WA, Church DF, Govindan CK, Crank G. Oxidation of thiols by nitric oxide and nitrogen dioxide: synthetic utility and toxicological implications. J Org Chem 1982;47:156–9.
[15] Wink DA, Nims RW, Darbyshire JF, et al. Reaction kinetics for nitrosation of cysteine and glutathione in aerobic nitric oxide solutions at neutral pH. Insights into the fate and physiological effects of intermediates generated in the NO/O_2 reaction. Chem Res Toxicol 1994;7:519–25.
[16] Gow AJ, Buerk DG, Ischiropoulos H. A novel reaction mechanism for the formation of S-nitrosothiol in vivo. J Biol Chem 1997;272: 2841–5.
[17] Wink DA, Darbyshire JF, Nims RW, Saavedra JE, 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 1993;6:23–7.
[18] Hogg N. The biochemistry and physiology of S-nitrosothiols. Annu Rev Pharmacol Toxicol 2002;42:585–600.
[19] Liu X, Miller MJ, Joshi MS, Thomas DD, 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 1998;95: 2175–9.
[20] Nedospasov A, Rafikov R, Beda N, Nudler E. An autocatalytic mechanism of protein nitrosylation. Proc Natl Acad Sci U S A 2000;97:13543–8.
[21] Arnelle DR, Stamler JS. NO^+, NO, and NO^- donation by S-nitrosothiols: implications for regulation of physiological functions by S-nitrosylation and acceleration of disulfide formation. Arch Biochem Biophys 1995;318:279–85.
[22] Hogg N. The kinetics of S-transnitrosation—a reversible second-order reaction. Anal Biochem 1999;272:257–62.
[23] Liu Z, Rudd MA, Freedman JE, Loscalzo J. S-transnitrosation reactions are involved in the metabolic fate and biological actions of nitric oxide. J Pharmacol Exp Ther 1998;284:526–34.
[24] Singh RJ, Hogg N, Joseph J, Kalyanaraman B. Mechanism of nitric oxide release from S-nitrosothiols. J Biol Chem 1996;271: 18596–603.
[25] Stamler JS, Toone EJ, Lipton SA, Sucher NJ. (S)NO signals: translocation, regulation, and a consensus motif. Neuron 1997;18:691–6.
[26] Ascenzi P, Colasanti M, Persichini T, et al. Re-evaluation of amino acid sequence and structural consensus rules for cysteine-nitric oxide reactivity. Biol Chem 2000;381:623–7.
[27] Pérez-Mato I, Castro C, Ruiz FA, Corrales FJ, Mato JM. Methionine adenosyltransferase S-nitrosylation is regulated by the basic and acidic amino acids surrounding the target thiol. J Biol Chem 1999; 274:17075–9.
[28] Williams JG, Pappu K, Campbell SL. Structural and biochemical studies of $p21^{Ras}$ S-nitrosylation and nitric oxide-mediated guanine nucleotide exchange. Proc Natl Acad Sci U S A 2003;100: 6376–81.
[29] Klatt P, Pineda Molina E, Lamas S. Nitric oxide inhibits c-Jun DNA binding by specifically targeted S-glutathionylation. J Biol Chem 1999;274:15857–64.
[30] Klatt P, Pineda Molina E, Pérez-Sala D, Lamas S. Novel application of S-nitrosoglutathione–Sepharose to identify proteins that are potential targets for S-nitrosoglutathione-induced mixed-disulphide formation. Biochem J 2000;349:567–78.
[31] Klatt P, Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem 2000;267:4928–44.
[32] Stamler JS, Lamas S, Fang FC. Nitrosylation: the prototypic redox-based signaling mechanism. Cell 2001;106:675–83.
[33] Mannick JB, Schonhoff CM. Nitrosylation: the next phosphorylation? Arch Biochem Biophys 2002;408:1–6.
[34] Lander HM, Ogiste JS, Pearce SFA, Levi R, Novogrodsky A. Nitric oxide-stimulated guanine nucleotide exchange on $p21^{ras}$. J Biol Chem 1995;270:7017–20.
[35] Sun J, Xin C, Eu JP, Stamler JS, Meissner G. Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. Proc Natl Acad Sci U S A 2001;98:11158–62.
[36] Nathan C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. J Clin Invest 2003;111:769–78.
[37] Schmidt HHHW, Hofmann H, Schindler U, et al. No NO from NO synthase. Proc Natl Acad Sci U S A 1996;93:14492–7.
[38] Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. J Biol Chem 2001;276:14533–6.
[39] Gaston B. Nitric oxide and thiol groups. Biochim Biophys Acta 1999;1411:323–33.
[40] Bredt DS. Nitric oxide signaling specificity—the heart of the problem. J Cell Sci 2003;116:9–15.
[41] Ziolo MT, Bers DM. The real estate of NOS signaling: location, location, location. Circ Res 2003;92:1279–81.
[42] Matsumoto A, Comatas KE, Liu L, Stamler JS. Screening for nitric oxide-dependent protein–protein interactions. Science 2003; 301:657–61.
[43] Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci U S A 2000;97:8841–8.
[44] Vanin AF, Malenkova IV, Serezhenkov VA. Iron catalyzes both decomposition and synthesis of S-nitrosothiols: optical and electron paramagnetic resonance studies. Nitric Oxide 1997;1:191–203.
[45] Mannick JB, Hausladen A, Liu L, et al. Fas-induced caspase denitrosylation. Science 1999;284:651–4.
[46] Hoffmann J, Haendeler J, Zeiher AM, Dimmeler S. TNFα and oxLDL reduce protein S-nitrosylation in endothelial cells. J Biol Chem 2001;276:41383–7.
[47] Mirza UA, Chait BT, Lander HM. Monitoring reactions of nitric oxide with peptides and proteins by electrospray ionization–mass spectrometry. J Biol Chem 1995;270:17185–8.
[48] Lander HM, Hajjar DP, Hempstead BL, et al. A molecular redox switch on $p21^{ras}$. Structural basis for the nitric oxide-$p21^{ras}$ interaction. J Biol Chem 1997;272:4323–6.
[49] Zech B, Wilm M, van Eldik R, Brüne B. Mass spectrometric analysis of nitric oxide-modified Caspase-3. J Biol Chem 1999; 274:20931–6.
[50] Kaneko R, Wada Y. Decomposition of protein nitrosothiolsin matrix-assisted laser desorption/ionization and electrospray ionization mass spectrometry. J Mass Spectrom 2003;38:526–30.
[51] Welch GN, Upchurch Jr GR, Loscalzo J. S-nitrosothiol detection. Methods Enzymol 1996;268:293–8.
[52] Wang K, Hou Y, Zhang W, et al. ^{15}N NMR and electronic properties of S-nitrosothiols. Bioorg Med Chem Lett 1999;9:2897–902.
[53] Fang K, Ragsdale NV, Carey RM, MacDonald T, Gaston B. Reductive assays for S-nitrosothiols: implications for measurements in biological systems. Biochem Biophys Res Commun 1998;252: 535–40.
[54] Yang BK, Vivas EX, Reiter CD, Gladwin MT. Methodologies for the sensitive and specific measurement of S-nitrosothiols, iron-nitrosyls, and nitrite in biological samples. Free Radic Res 2003;37:1–10.
[55] Wink DA, Kim S, Coffin D, et al. Detection of S-nitrosothiols by fluorometric and colorimetric methods. Methods Enzymol 1999;301: 201–11.

[56] Kostka P, Park JK. Fluorometric detection of S-nitrosothiols. Methods Enzymol 1999;301:227–35.
[57] Mannick JB, Schonhoff C, Papeta N, et al. S-nitrosylation of mitochondrial caspases. J Cell Biol 2001;154:1111–6.
[58] Schonhoff CM, Gaston B, Mannick JB. Nitrosylation of cytochrome *c* during apoptosis. J Biol Chem 2003;278:18265–70.
[59] Matsushita K, Morrell CN, Cambien B, et al. Nitric oxide regulates exocytosis by S-nitrosylation of N-ethylmaleimide-sensitive factor. Cell 2003;115:139–50.
[60] Jaffrey SR, Erdjument-Bromage H, Ferris CD, Tempst P, Snyder SH. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol 2001;3:193–7.
[61] Kuncewicz T, Sheta EA, Goldknopf IL, Kone BC. Proteomic analysis of S-nitrosylated proteins in mesangial cells. Mol Cell Proteomics 2003;2:156–63.
[62] Martínez-Ruiz A, Lamas S. Detection and proteomic identification of S-nitrosylated proteins in endothelial cells. Arch Biochem Biophys 2004;423:193–200.
[63] Stamler JS, Lamas S, Fang FC. Cell 2001: http://www.cell.com/cgi/content/full/106/106/675/DC101/.
[64] Mott HR, Carpenter JW, Campbell SL. Structural and functional analysis of a mutant Ras protein that is insensitive to nitric oxide activation. Biochemistry 1997;36:3640–4.
[65] Janssen-Heininger YM, Poynter ME, Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. Free Radic Biol Med 2000;28:1317–27.
[66] Matthews JR, Wakasugi N, Virelizier JL, Yodoi J, Hay RT. Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulphide bond involving cysteine 62. Nucleic Acids Res 1992;20:3821–30.
[67] Hayashi T, Ueno Y, Okamoto T. Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin. J Biol Chem 1993;268:11380–8.
[68] Pineda-Molina E, Klatt P, Vázquez J, et al. Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding. Biochemistry 2001;40:14134–42.
[69] Matthews JR, Botting CH, Panico M, Morris HR, Hay RT. Inhibition of NF-kappaB DNA binding by nitric oxide. Nucleic Acids Res 1996;24:2236–42.
[70] DelaTorre A, Schroeder RA, Kuo PC. Alteration of NF-kappa B p50 DNA binding kinetics by S-nitrosylation. Biochem Biophys Res Commun 1997;238:703–6.
[71] DelaTorre A, Schroeder RA, Bartlett ST, Kuo PC. Differential effects of nitric oxide-mediated S-nitrosylation on p50 and c-jun DNA binding. Surgery 1998;124:137–42.
[72] Bogdan C. Nitric oxide and the regulation of gene expression. Trends Cell Biol 2001;11:66–75.
[73] Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. Biochemistry 2001;40:1688–93.
[74] DelaTorre A, Schroeder RA, Punzalan C, Kuo PC. Endotoxin-mediated S-nitrosylation of p50 alters NF-kappaB-dependent gene transcription in ANA-1 murine macrophages. J Immunol 1999;162:4101–8.
[75] Park SK, Lin HL, Murphy S. Nitric oxide regulates nitric oxide synthase-2 gene expression by inhibiting NF-kappaB binding to DNA. Biochem J 1997;322:609–13.
[76] Marshall HE, Stamler JS. Nitrosative stress-induced apoptosis through inhibition of NF-kappaB. J Biol Chem 2002;277:34223–8.
[77] Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000;88:1474–80.
[78] Liu Y, Christou H, Morita T, et al. Carbon monoxide and nitric oxide suppress the hypoxic induction of vascular endothelial growth factor gene via the 5′ enhancer. J Biol Chem 1998;273:15257–62.
[79] Sogawa K, Numayama-Tsuruta K, Ema M, et al. Inhibition of hypoxia-inducible factor 1 activity by nitric oxide donors in hypoxia. Proc Natl Acad Sci U S A 1998;95:7368–73.
[80] Kimura H, Weisz A, Kurashima Y, et al. Hypoxia response element of the human vascular endothelial growth factor gene mediates transcriptional regulation by nitric oxide: control of hypoxia-inducible factor-1 activity by nitric oxide. Blood 2000;95:189–97.
[81] Palmer LA, Gaston B, Johns RA. Normoxic stabilization of hypoxia-inducible factor-1 expression and activity: redox-dependent effect of nitrogen oxides. Mol Pharmacol 2000;58:1197–203.
[82] Wang F, Sekine H, Kikuchi Y, et al. HIF-1α-prolyl hydroxylase: molecular target of nitric oxide in the hypoxic signal transduction pathway. Biochem Biophys Res Commun 2002;295:657–62.
[83] Metzen E, Zhou J, Jelkmann W, Fandrey J, Brüne B. Nitric oxide impairs normoxic degradation of HIF-1α by Inhibition of prolyl hydroxylases. Mol Biol Cell 2003;14:3470–81.
[84] Brüne B, Zhou J. The role of nitric oxide (NO) in stability regulation of hypoxia inducible factor-1a (HIF-1α). Curr Med Chem 2003;10:845–55.
[85] Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen in hypoxia by nitric oxide: effect on HIF1α. Science 2003;302:1975–8.
[86] Mateo J, García-Lecea M, Cadenas S, Hernández C, Moncada S. Regulation of hypoxia-inducible factor-1alpha by nitric oxide through mitochondria-dependent and -independent pathways. Biochem J 2003;376:537–44.
[87] Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. Science 2002;295:858–61.
[88] Sumbayev VV, Budde A, Zhou J, Brüne B. HIF-1α protein as a target for S-nitrosation. FEBS Lett 2003;535:106–12.
[89] Yasinska IM, Sumbayev VV. S-nitrosation of Cys-800 of HIF-1α protein activates its interaction with p300 and stimulates its transcriptional activity. FEBS Lett 2003;549:105–9.
[90] Lando D, Pongratz I, Poellinger L, Whitelaw ML. A redox mechanism controls differential DNA binding activities of hypoxia-inducible factor (HIF) 1α and the HIF-like factor. J Biol Chem 2000;275:4618–27.
[91] Kröncke K.-D.. Zinc finger proteins as molecular targets for nitric oxide-mediated gene regulation. Antioxid Redox Signal 2001;3:565–75.
[92] Berendji D, Kolb-Bachofen V, Zipfel PF, et al. Zinc finger transcription factors as molecular targets for nitric oxide-mediated immunosuppression: inhibition of IL-2 gene expression in murine lymphocytes. Mol Med 1999;5:721–30.
[93] Kröncke KD, Carlberg C. Inactivation of zinc finger transcription factors provides a mechanism for a gene regulatory role of nitric oxide. FASEB J 2000;14:166–73.
[94] Galigniana MD, Piwien-Pilipuk G, Assreuy J. Inhibition of glucocorticoid receptor binding by nitric oxide. Mol Pharmacol 1999;55:317–23.
[95] Wang S, Wang W, Wesley RA, Danner RL. A Sp1 binding site of the tumor necrosis factor alpha promoter functions as a nitric oxide response element. J Biol Chem 1999;274:33190–3.
[96] Maret W. The function of zinc metallothionein: a link between cellular zinc and redox state. J Nutr 2000;130:1455S–8S.
[97] Berendji D, Kolb-Bachofen V, Meyer KL, et al. Nitric oxide mediates intracytoplasmic and intranuclear zinc release. FEBS Lett 1997;405:37–41.
[98] Pearce LL, Gandley RE, Han W, et al. Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent fusion protein. Proc Natl Acad Sci U S A 2000;97:477–82.
[99] Chen Y, Irie Y, Keung WM, Maret W. S-nitrosothiols react preferentially with zinc thiolate clusters of metallothionein III through transnitrosation. Biochemistry 2002;41:8360–7.
[100] Zaragoza C, Soria E, López E, et al. Activation of the mitogen activated protein kinase extracellular signal-regulated kinase 1 and 2 by the nitric oxide-cGMP-cGMP-dependent protein kinase axis regulates the expression of matrix metalloproteinase 13 in vascular endothelial cells. Mol Pharmacol 2002;62:927–35.
[101] Zaragoza C, Balbín M, López-Otín C, Lamas S. Nitric oxide regu-

lates matrix metalloprotease-13 expression and activity in endothelium. Kidney Int 2002;61:804–8.
[102] Gurjar MV, DeLeon J, Sharma RV, Bhalla RC. Mechanism of inhibition of matrix metalloproteinase-9 induction by NO in vascular smooth muscle cells. J Appl Physiol 2001;91:1380–6.
[103] Upchurch J, Gilbert R, Ford JW, Weiss SJ, et al. Nitric oxide inhibition increases matrix metalloproteinase-9 expression by rat aortic smooth muscle cells in vitro. J Vasc Surg 2001;34:76–83.
[104] Eagleton MJ, Peterson DA, Sullivan VV, et al. Nitric oxide inhibition increases aortic wall matrix metalloproteinase-9 expression. J Surg Res 2002;104:15–21.
[105] Gu Z, Kaul M, Yan B, et al. S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 2002; 297:1186–90.
[106] Meszaros LG, Minarovic I, Zahradnikova A. Inhibition of the skeletal muscle ryanodine receptor calcium release channel by nitric oxide. FEBS Lett 1996;380:49–52.
[107] Zahradnikova A, Minarovic I, Venema RC, Meszaros LG. Inactivation of the cardiac ryanodine receptor calcium release channel by nitric oxide. Cell Calcium 1997;22:447–54.
[108] Stoyanovsky D, Murphy T, Anno PR, Kim YM, Salama G. Nitric oxide activates skeletal and cardiac ryanodine receptors. Cell Calcium 1997;21:19–29.
[109] Xu L, Eu JP, Meissner G, Stamler JS. Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science 1998;279:234–7.
[110] Suko J, Drobny H, Hellmann G. Activation and inhibition of purified skeletal muscle calcium release channel by NO donors in single channel current recordings. Biochim Biophys Acta 1999;1451: 271–87.
[111] Hart JD, Dulhunty AF. Nitric oxide activates or inhibits skeletal muscle ryanodine receptors depending on its concentration, membrane potential and ligand binding. J Membr Biol 2000;173:227–36.
[112] Eu JP, Sun J, Xu L, Stamler JS, Meissner G. The skeletal muscle calcium release channel: coupled O_2 sensor and NO signaling functions. Cell 2000;102:499–509.
[113] Sun J, Xu L, Eu JP, Stamler JS, Meissner G. Classes of thiols that influence the activity of the skeletal muscle calcium release channel (RyR1). J Biol Chem 2001;276:15625–30.
[114] Sun J, Xu L, Eu JP, Stamler JS, Meissner G. Nitric oxide, NOC-12, and S-nitrosoglutathione modulate the skeletal muscle calcium release channel/ryanodine receptor by different mechanisms. An allosteric function for O_2 in S-nitrosylation of the channel. J Biol Chem 2003;278:8184–9.
[115] Aracena P, Sánchez G, Donoso P, Hamilton SL, Hidalgo C. S-glutathionylation decreases Mg^{2+}inhibition and S-nitrosylation enhances Ca^{2+} activation of RyR1 channels. J Biol Chem 2003;278: 42927–35.