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European Journal of Pharmacology 429 (2001) 279–286

ejp

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Review

Nitric oxide and mechanisms of redox signalling: matrix and matrix-metabolizing enzymes as prime nitric oxide targets

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Accepted 27 July 2001

Abstract

One of the greatest biomedical breakthroughs of the twentieth century was the discovery of endothelium-derived relaxing factor and its identification as nitric oxide (NO). NO has received special attention ever since: besides its potent vasodilatory and vasoprotective effects, NO was identified as a key player in innate immunity and was found to act as an unconventional type of neurotransmitter. This article focuses on mechanisms of NO signalling that form the basis of functional cell responses to accommodate changes in the cellular microenvironment. Redox-based regulation of signal transduction and, on a more long-term scale, changes in gene expression will be exemplified by NO-modulation of matrix components and matrix-metabolizing enzymes. It seems to be a safe bet that ongoing analyses of NO signalling and gene expression will provide a wealth of promising therapeutic targets in human diseases.

Keywords: Nitric oxide; Redox signalling; Matrix-metabolizing enzyme

1. Introduction

Nitric oxide (NO) is a diffusible and short-lived free radical gas that can be generated in mammalian cells by a family of NO synthases (NOS). This family comprises the constitutively expressed neuronal NOS (nNOS) and endothelial NOS (eNOS), and the cytokine-inducible NOS (iNOS). iNOS requires a delay of several hours before the onset of NO production but, once induced, this enzyme is active for hours to days and produces NO in 1000-fold larger quantities than the constitutive enzymes eNOS and nNOS (Moncada et al., 1991; Beck et al., 1999). Under physiological conditions cells produce only minute amounts of NO by the constitutive enzymes and only trace amounts of reactive oxygen species are available to scavenge NO, thus indicating that direct NO chemistry will dominate functional cell responses (Grisham et al., 1999). The physiologically most relevant action of NO is the activation of the soluble guanylate cyclase by binding to the enzyme's haem moiety. The subsequent increase in cyclic GMP concentrations alters the activity of several target proteins (see below). In a similar way the formation of nitrosyl complexes affects other metalloproteins such as catalase, cytochrome oxidase, cytochrome *P*450 and NOS itself. Moreover, NO can scavenge superoxide and other free radicals and also inhibit superoxide-driven Fenton chemistry and lipid peroxidation and thus, may depict quite remarkable antioxidant features. In contrast, large amounts of NO as produced by iNOS in an inflammatory setting, often accompanied by a co-generation of reactive oxygen species will shift NO chemistry towards indirect effects such as nitrosation, nitration and oxidation (Grisham et al., 1999). The interaction of NO with molecular oxygen or superoxide will cause the generation of the potent nitrosating agent N_2O_3 and peroxynitrite, respectively. Furthermore, *S*-nitrosothiol adducts are formed by the interaction between N_2O_3 and certain protein thiol groups and trigger signalling through alterations of protein kinases and phosphatases, G-proteins and ion channels (see below). Finally, these signalling devices directly or indirectly modulate the activity of transcription factors and thus alter gene expression which constitute a fundamental cause of disease-associated pathophysiologies. A detailed discussion of regulation of gene expression by NO is provided elsewhere (Marshall et al., 2000; Bogdan, 2001; Pfeilschifter et al., 2001).

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01326-7

2. Soluble guanylate cyclase and cyclic GMP as mediators of NO action

The physiologically most relevant action of NO is the activation of soluble guanylate cyclase by nitrosation of its haem moiety (Ignarro, 1990). The subsequent increase in cyclic GMP level alters the activity of three main target proteins: (i) cyclic GMP-regulated ion channels, (ii) cyclic GMP-regulated phosphodiesterases, and (iii) cyclic GMP-dependent protein kinases (for a review, see Schmidt et al., 1993).

The molecular basis of the sensory systems of visualisation and olfaction is related to the action of cation channels which are regulated by cellular cyclic GMP levels. These proteins have one binding site for cyclic GMP. Binding of cyclic GMP critically determines the function of these sensors. Activation of the vertebrate rod photoreceptor cells by light results in hydrolysis of cyclic GMP. The drop in cellular cyclic GMP concentration leads to closure of a cyclic GMP-regulated cation channel with subsequent hyperpolarisation of the cell; this is the primary neuronal response in visualisation (Dhallan et al., 1992). Activation of odorant receptors in olfactory cilia is likely to be connected to an inositoltrisphosphate-mediated rise in intracellular Ca^{2+} concentration and activation of a constitutive NOS. NO increases cyclic GMP levels in adjacent neurones, which in turn mediates further activation of the odorant signalling pathway by opening Ca^{2+} channels (Breer and Shepherd, 1993).

Phosphodiesterases play a key role in controlling the actions of the second messengers cyclic AMP and cyclic GMP. Of the six family members of phosphodiesterases, the enzyme activity of type II and type III proteins is directly regulated by binding of cyclic GMP to conserved non-catalytic cyclic GMP-binding domains. Type II phosphodiesterases are stimulated by cyclic GMP binding, whereas the type III enzymes are inhibited by cyclic GMP. Therefore, cyclic GMP can increase cyclic AMP levels via inhibition of type III phosphodiesterase. In contrast, activation of type II enzymes increases cyclic AMP hydrolysis and accordingly decreases cyclic AMP concentrations (Schmidt et al., 1993).

Two major classes of cyclic GMP-dependent protein kinases have been identified, the soluble type I (GKI) and the membrane-bound type II (GKII) enzymes. GKI is further subdivided in α and β isoforms. GKI exists as a dimer, whereas GKII is a monomer. Protein analysis of GKI revealed a dimerisation domain which includes an autophosphorylation and an autoinhibitory site, two cyclic GMP-binding domains and a catalytic domain. GKII is predominantly expressed in the intestinal epithelial brush border and is involved in intestinal Cl^- absorption and secretion. Moreover, GKII was also detected in other tissues including mouse brain and rat kidney (Eigenthaler et al., 1999). The membrane targeting of GKII is required for some of its biological activities and is, at least in part, mediated by N-terminal myristoylation of the protein (Vaandrager et al., 1998). GKI is expressed in various tissues, and its role in physiology is much better characterized. In myocytes, activation of GKI modulates contractility by inhibiting an inward Ca^{2+} current. In the kidney, GKI is expressed in different cell types such as mesangial cells, smooth muscle cells, microvascular pericytes and myofibroblasts. Functionally, it has been demonstrated that GKI regulates an amiloride-sensitive Na^+ channel in the inner medullary collecting duct. In the cerebellum, GKI is highly expressed in Purkinje cells. However, its role in these cells is not yet clear. Furthermore, in smooth muscle cells and platelets, GKI inhibits agonist-induced elevations in Ca^{2+} concentration and platelet activation (Schmidt et al., 1993).

Only a minor part of the genes regulated by NO seem to be targeted by cyclic GMP-triggered signalling mechanisms (Pfeilschifter et al., 2001). However, both activation and inhibition of gene expression by cyclic GMP has been reported. In many cases, cyclic GMP regulates gene expression at the transcriptional level, but posttranscriptional modes of regulation by cyclic GMP have also been observed (Eigenthaler et al., 1999).

3. Protein kinases and phosphatases as targets of NO actions

The first evidence for an effect of NO on protein kinase cascades was presented by Lander et al. (1993a), who found that, in human peripheral blood mononuclear cells, NO-generating compounds stimulated a membrane-associated protein tyrosine phosphatase activity which led to a dephosphorylation and activation of the Src family protein tyrosine kinase $p56^{lck}$, which is critically involved in T cell activation. Later on, NO was reported to control Src kinase activity through *S*-nitrosation and subsequent disulfide formation which, in turn, destabilises the Src structure for tyrosine-416 autophosphorylation and activation (Akhand et al., 1999).

Lander et al. (1996) also showed that NO activates all three parallel mitogen-activated protein kinase (MAPK) cascades (Fig. 1) in Jurkat cells, i.e. the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) cascade, the stress-activated p38-MAPK cascade and the classical extracellular signal-regulated kinase (ERK)/MAPK cascade. A similar stimulatory effect of NO on the different MAPK cascades was observed in glomerular mesangial cells and endothelial cells (Pfeilschifter and Huwiler, 1996; Callsen et al., 1998; Huwiler and Pfeilschifter, 1999). Mechanistically, activation of the different MAPK cascades by NO may occur either by direct interaction with the kinases themselves or by modulation of upstream factors such as the JNK kinase (JNKK1/SEK1)(Kim et al., 1997) or the small GTP-binding protein $p21^{ras}$ (Lander et al., 1995; Yun et al., 1998). The activation of $p21^{ras}$ by

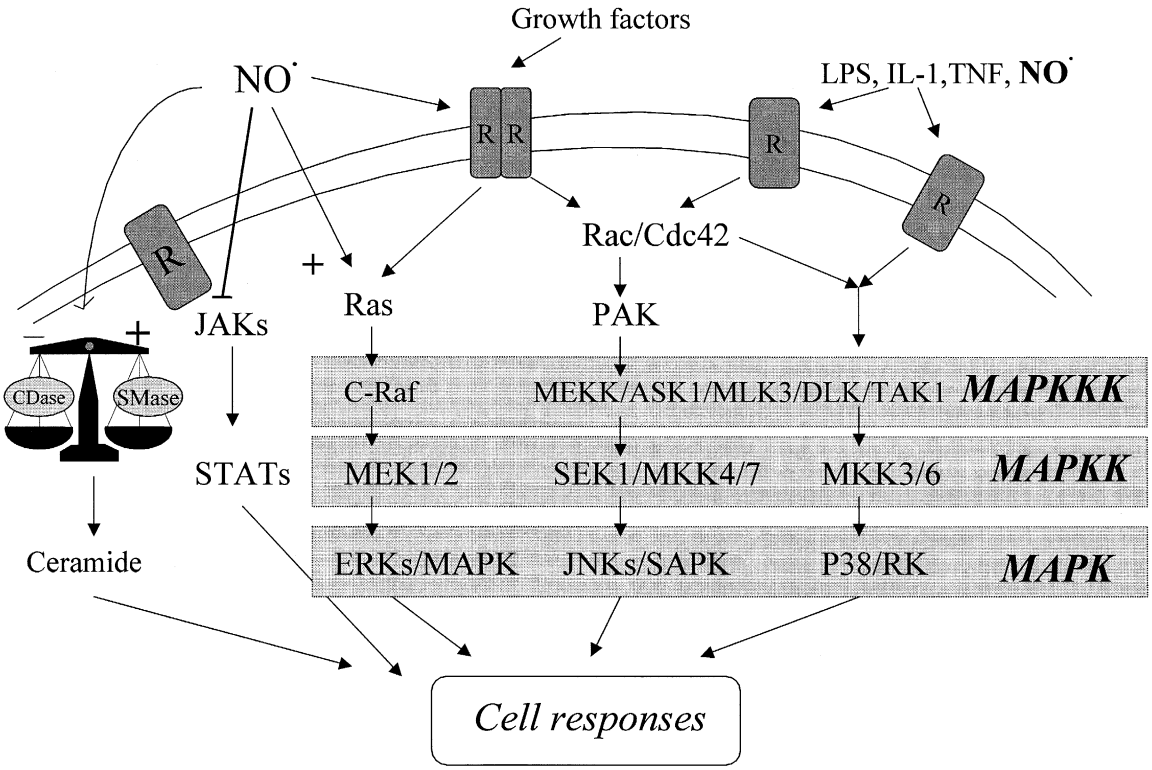

Fig. 1. Involvement of NO in cell signalling cascades. ASK, apoptosis signal-regulating kinase; ERK, extracellular signal-regulated kinase; CDase, ceramidase; DLK, dual leucine zipper kinase; IL-1, interleukin-1; JAK, janus kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MKK, MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; MEK, MAPK kinase; MEK, mitogen-activated and ERK kinase; MLK, mixed lineage kinase; NO, nitric oxide; PAK, p21-activated kinase; R, receptor; RK, reactivating kinase; SAPK, stress-activated protein kinase; SMase, sphingomyelinase; STAT, signal transducer and activator of transcription; TAK, transforming growth factor-activated kinase; TNF, tumour necrosis factor-α.

NO was reported to be direct, reversible and initiated by *S*-nitrosation of a critical cysteine residue of $p21^{ras}$ which stimulates guanine nucleotide exchange. In this way NO mimics the action of guanine nucleotide exchange factors (Lander et al., 1995). A well-established downstream target of $p21^{ras}$ is phosphatidylinositol 3′-kinase which, indeed, was found to be recruited by redox-activated Ras and subsequently activated in Jurkat T cells (Deora et al., 1998). In contrast, NO inhibits thrombin receptor-activating peptide-induced phosphatidylinositol 3′-kinase activity in human platelets (Pigazzi et al., 1999). Other potential direct targets for NO and upstream factors in the MAPK cascades are the heterotrimeric G-proteins. NO greatly enhances GTPase activity of Gs_α and Gi_α both in T cells and in vitro when pure recombinant proteins were used (Lander et al., 1993b). Alternatively, NO may directly modulate members of the different MAPK cascades and cause either activation or inhibition of the enzymes. The NO-releasing compound *S*-nitrosoglutathione was found to cause *S*-nitrosation and inactivation of the JNK2/SAPK cascade in vitro and may thereby exert its anti-apoptotic effect in B-lymphocytes (So et al., 1998). In addition, NO has been reported to inhibit JNK1 activation in murine microglial cells and macrophages via *S*-nitrosation of cysteine-116 (Park et al., 2000). Similarly, the NO-generating compound diethylenetriamine nitric oxide inhibits JNK activation in phaeochromocytoma (PC12) cells (Park et al., 1996). However, future studies regarding the effect of NO on JNK activity must confirm that this signalling module is indeed inhibited by *S*-nitrosation in vivo.

Another family of protein kinases that plays a key role in several signal-transducing pathways is the family of protein kinase C isoenzymes. Reactive oxygen species as well as NO have been reported to increase protein kinase C activity (Klann et al., 1998). In contrast, NO was found to suppress protein kinase C activity in mesangial cells (Gopalakrishna et al., 1993; Studer et al., 1996). However, neither of these studies gave any evidence for a direct effect of NO on the activation state of protein kinase C or did address the involvement of distinct isoenzymes.

Recently, an additional class of protein kinases was shown to be directly modulated by NO in vitro and in vivo, the Janus kinases (JAKs), including the Tyrosine kinases (TYKs). Both JAK2 and JAK3 autokinase activities were found to be inhibited by NO, presumably by oxidation of crucial dithiols to disulphides (Duhe et al., 1998). Such an inhibition of JAK activity in vivo may contribute to the immunosuppressive effects of NO be-

cause JAKs transmit signals from almost all types of cytokine receptors. These negative regulatory actions of NO contrast with a report that identifies iNOS-derived NO as an indispensable and positive signal in the interleukin 12-mediated triggering of natural killer (NK) cells during the innate response to the protozoan parasite *Leishmania*. This latter effect of NO is selective, because it pertains to NK cells which do express iNOS, but not to T cells which lack iNOS. Moreover, it is a prerequisite for TYK2 activation, but not for JAK1 and JAK2 (Diefenbach et al., 1999). However, the molecular mechanism of NO action on either JAK or TYK remains to be elucidated.

Other possible targets for NO represent the family of protein phosphatases. By direct interaction with this class of enzymes, either an activation or inhibition of phosphatase activity may be achieved, and this will lead to increased or decreased phosphorylation of their target proteins which include, importantly, members of the different MAPK pathways. Activation of membrane-bound phosphatase was reported in T cells (Lander et al., 1993a). Furthermore, protein tyrosine phosphatase activation was also observed in rat aortic smooth muscle cells (Kaur et al., 1998). In contrast, NO was reported to inactivate a low molecular mass protein tyrosine phosphatase in vitro by *S*-nitrosation of two adjacent cysteine residues in the active site of the enzyme (Caselli et al., 1994). Comparable data were described recently for mesangial cells: NO was found to inhibit directly a tyrosine phosphatase activity, leading to increased phosphorylation and activation of the classical MAPKs (Callsen et al., 1998). However, the identity of this tyrosine phosphatase was not analysed further. Moreover, it has been reported that NO leads to an induction of MAPK phosphatase-1 (MKP-1) mRNA in human embryonic lung fibroblasts, which would explain an increased MKP activity and conversely, a decreased MAPK activity in these cells (Marquis and Demple, 1998). In addition, NO was reported to down-regulate MKP-3 mRNA and protein in human umbilical vein endothelial cells (Rossig et al., 1999). However, the mechanistic details and the physiological relevance of the latter observation remains to be shown. NO was also observed to inhibit platelet-derived growth factor phosphotyrosine phosphatases in rat renal mesangial cells, an action that was not mimicked by cyclic GMP analogues (Callsen et al., 1999). In a similar manner NO was found to block epidermal growth factor tyrosine phosphatase activities in HER14 cells and thus stimulates tyrosine phosphorylation of the receptor even in the absence of epidermal growth factor (Peranovich et al., 1995). In contrast, Estrada et al. (1997) showed that NO reversibly inhibits epidermal growth factor receptor tyrosine kinase in fibroblasts and that this phenomenon correlated well with the anti-proliferative effect of NO in the same cells. Despite these apparently discrepant findings it is obvious that protein phosphatases are targets of NO and reactive oxygen species and may sense the cellular redox state. All tyrosine phosphatases have a common active site that comprises a HCX_5R sequence motif, and a catalytic mechanism that involves the formation of a transient phosphoenzyme intermediate to the conserved cysteine residue. Protein serine/threonine phosphatases, like protein phosphatase 1, 2A, 2B (calcineurin) and 2C, are metalloenzymes that are also sensitive to redox regulation (Rusnak and Reiter, 2000). In this context, various challenges still need to be met, most importantly the elucidation of the detailed mechanisms and functional significance of redox regulation of protein phosphatases under in vivo conditions.

In conclusion, all these studies suggest that NO can deliver signals into all the major MAPK cascades including the classical ERK, SAPK/JNK and p38-MAPK cascades as well as the JAK/signal transducer and activator of transcription (STAT) pathway. An integration of signals will lead to cell type-specific responses that critically depend on interactions between the individual constituents of a cell and the components of the different signalling cascades. These cascade events then trigger the phosphorylation of key nuclear proteins, including transcription factors like c-Jun, ternary complex factors or STATs and, finally, lead to alterations in gene expression (Pfeilschifter et al., 2001). In this way NO helps to orchestrate spatial and temporal patterns of gene transcription and it is the combination of altered cellular functions that constitutes the appropriate physiological response. In this context, it is worth mentioning that besides the above described rapid responses initiated by NO, there is also a set of signals generated by NO in a more delayed fashion. The first evidence for ceramide generation by NO was presented by Huwiler et al. (1999a,b). The lipid signalling molecule ceramide is generated by sphingomyelin hydrolysis and was identified as a key player in stress signalling in mammalian cells (Huwiler et al., 2000; Pfeilschifter and Huwiler, 2000). Exposure of glomerular endothelial cells and mesangial cells to NO donors caused a delayed and sustained up-regulation of ceramide levels by activating sphingomyelinases and concomitantly inhibiting ceramidases, and particularly the late-phase ceramide generation may be responsible for the further processing of a pro-apoptotic signal (Huwiler et al., 1999a,b). Ceramide, in turn, has been demonstrated to target important signalling devices in cells like the protein kinase c-Raf (Huwiler et al., 1996), protein kinase C isoenzymes α and δ (Huwiler et al., 1998), cytosolic phospholipase A_2 (Huwiler et al., 2001) and others (for review, see Huwiler et al., 2000) and, thus may trigger a second wave of signals originating from these enzymes. NO-induced ceramide generation has been confirmed in human leukemia HL-60 cells (Takeda et al., 1999), whereas in human monocytic U937 cells, NO was reported to reduce tumour necrosis factor α (TNF-α)-induced ceramide production in a cyclic GMP-dependent manner (De Nadai et al., 2000), reminiscent of NO inhibition of growth factor-elicited phosphoinositide turnover and calcium signalling (Clementi et al., 1995).

4. Matrix and matrix-metabolizing enzymes as targets of NO

NO derived from iNOS seems to contribute to the pathophysiology of inflammatory diseases throughout the body, but its role goes beyond cell and tissue damage. The inflammatory response is a dynamic set of events that is tightly regulated and comprises an initial production and release of pro-inflammatory mediators in the affected area to recruit immune cells for clearing harmful pathogens. This is followed by an anti-inflammatory phase that orchestrates a sophisticated orderly process of repair for resolution of disease (Pfeilschifter, 1994). The aberrant production of either class of mediators, may lead to the irreversible alteration of tissue architecture and initiate a chronic inflammatory and fibrogenic response, a hallmark of a variety of disorders including atherosclerosis, renal and pulmonary fibrosis (Kovacs, 1991; Kitamura and Fine, 1999). During the last few years, an increasing number of genes have been shown to be under the regulatory control of NO, including pro-inflammatory mediators as well as a set of gene products that prevent or repair cell and tissue damage (Pfeilschifter, 2001; Pfeilschifter et al., 2001). One key facet of this gene regulatory activity may be the control of iNOS induction itself.

Indeed, one of the first genes identified as a target for transcriptional regulation by NO was iNOS, suggesting that NO modulates its own biosynthetic machinery. Inhibition of NO synthesis clearly reduced interleukin 1β-stimulated iNOS expression, suggesting that NO functions in a positive feedback loop that speeds up and strengthens its own biosynthesis (Mühl and Pfeilschifter, 1995). This potent amplification mechanism may form the basis for the excessive formation of NO in acute and chronic inflammatory diseases. The expression of iNOS in mammalian cells is governed predominantly by the transcription factor, NFκB, which regulates the expression of many inflammatory mediators (Janssen-Heininger et al., 2000). Intriguingly, NO has been reported to exert a biphasic regulation of NFκB activity which crucially depends on the local concentration of NO. At earlier time points NO augments cell activation, producing a sharp rise in protein expression, while at later time points, NO has an inhibitory effect on cells, such that a termination of activation is brought about (Connelly et al., 2001).

NO up- or down-regulates a heterogeneous set of gene products including protective mediators, pro-inflammatory mediators, chemokines and cytokines, adhesion molecules, growth factors, hormones, receptors and signalling devices (for a review, see Pfeilschifter et al., 2001). Many of the genes targeted by NO share roles in common physiological or pathophysiological processes. For sure, one of the preferred targets of NO regulation comprises extracellular matrix proteins and their metabolizing enzymes, the matrix

Table 1
Compilation of NO-regulated matrix components, matrix-metabolizing enzymes and cytokines

Gene product	Up- or down-regulation	cGMP-dependent	Cells/organ	References
Collagen	↑	n.d.	Mesangial cells	Trachtman et al., 1995
	↓	yes	Dermal fibroblast	Chu and Prasad, 1999
	↓	no	Mesangial cells	Craven et al., 1997
	↓	n.d.	Kidney	Shihab et al., 2000
	↓	n.d.	Kidney	Tharaux et al., 1999
Fibronectin	↑	n.d.	Mesangial cells	Trachtman et al., 1995
	↓	n.d.	Kidney	Shihab et al., 2000
Laminin	↑	n.d.	Mesangial cells	Trachtman et al., 1995
Biglycan	↓	n.d.	Kidney	Shihab et al., 2000
SPARC	↓	no	Mesangial cells, Kidney	Walpen et al., 2000
MMP-2	↑	n.d.	Synovial cells	Hirai et al., 2001
	↓	yes	Tumor cells	Jurasz et al., 2001
MMP-9	↓	no	Mesangial cells	Eberhardt et al., 1999; Eberhardt et al., 2000
MMP-13	↑	yes	Endothelial cells	Zaragoza et al., 2002
	↑	n.d.	Chondrocytes	Sasaki et al., 1998
TIMP-1	↓	no	Mesangial cells	Eberhardt et al., 1999
t-PA	↓	no	Mesangial cells	Eberhardt et al., 2002
PAI-1	↓	yes	Mesangial cells	Eberhardt et al., 2002
	↓	yes	Vascular smooth muscle cells	Bouchie et al., 1998
Elastases	↓	yes	Vascular smooth muscle cells	Mitani et al., 2000
TGFβ1	↓	no	Mesangial cells	Craven et al., 1997
			Kidney	Shihab et al., 2000
			Chondrocytes	Studer et al., 1999
	↑	n.d.	Kidney	Yang et al., 1998
bFGF	↑	n.d.	Chondrocytes	sasaki et al., 1998

BFGF, basic fibroblast growth factor; SPARC, secreted protein acidic and rich in cysteine; MMP, matrix metalloproteinase; PAI, plasminogen activator inhibitor; TGFβ, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; t-PA, tissue plasminogen activator.

metalloproteinases and plasminogen activators, and their endogenous inhibitors. In kidney, lung, heart, and liver accumulation of extracellular matrix is often a hallmark of chronic disease, eventually leading to the development of sclerosis (Fogo, 2001). In this context, a coordinate expression of proteases and their inhibitors by inflammatory cytokines and NO will allow a fine-tuned regulation of tissue proteolysis and protect against overwhelming tissue destruction. NO also modulates the expression of major matrix components such as fibronectin, collagen or laminin, which may also be important for tissue remodelling in chronic inflammatory diseases. Table 1 provides a compilation of NO targets related to matrix components, matrix-metabolizing enzymes and fibrogenic cytokines. The most convincing demonstration, also in terms of in vivo relevance, of NO-regulated expression of a matrix constituent, is SPARC (secreted protein acidic and rich in cysteine, also known as BM-40 or osteonectin) which was inhibited by NO in renal mesangial cells and during endotoxemia in the rat kidney (Walpen et al., 2000). The highly glycosylated SPARC protein shows a variety of biological activities (Lane and Sage, 1994), and its action as a scavenger of platelet-derived growth factor may be of relevance in the course of glomerulonephritis (Pichler et al., 1996). By modulating SPARC expression, NO may subsequently affect mesangial cell proliferation in the course of glomerular inflammation. Cross-communication with other pro-oxidant or anti-oxidant mediators will critically influence the final outcome under pathological conditions when iNOS is expressed. Besides NO, superoxide is another inflammatory mediator synthesised by different enzymes. Once primed and activated by pro-inflammatory cytokines such as interleukin 1β and TNF-α, most cells co-produce NO and superoxide. Reactive oxygen species themselves are potent modulators of signal transduction cascades and subsequent gene expression (Allen and Tresini, 2000). In this context, it is important to note that both radicals have opposite effects on matrix metalloproteinase 9 expression, with superoxide amplifying cytokine-stimulated matrix metalloproteinase 9 expression, and NO exerting an inhibitory effect (Eberhardt et al., 2000).

It is likely that some of the apparent controverse data depicted in Table 1 may reflect timing differences in the generation of both types of radicals in the in vitro and in vivo situation. A dynamic temporal organisation of the NO and superoxide signals and the ability of a cell to spatially resolve both signals may help to clarify these controverse reports.

Another exciting aspect of NO modulation of cell–matrix interaction is the potential of NO to cause redox rearrangement within cysteine-rich domains of integrin adhesion receptors that contain an on/off switch. In this way, ligand binding affinity to integrins is regulated (Yan and Smith, 2000) and the interaction of mast cells with fibronectin has been found to be modulated by NO (Wills et al., 1999).

5. Perspectives

Much of the charm in the investigation of NO functions has been in discovering its novel and unexpected roles in health and disease, particularly the more recent appreciation of NO's capability in regulating gene expression (Marshall et al., 2000; Bogdan, 2001; Pfeilschifter et al., 2001). Whatever the details, it is quite clear that a critical regulatory role in inflammatory gene expression is played by NO. One preferred target of NO gene regulation comprises matrix components and matrix-metabolising enzymes as outlined above. In contrast to the extensive amount of research on factors promoting matrix synthesis, key players of increased matrix degradation, which would allow for resolution of fibrotic diseases, have not been well studied. In this context it is worth to highlight that also protease inhibitors are targeted by NO (Eberhardt et al., 1999, 2000, in press). In terms of therapeutic approaches, pharmacological inhibition of local pathogenetic components is one way, and reinforcement of endogenous defense strategies is an alternative way to the treatment of fibrotic diseases.

Acknowledgements

The authors' work was supported by the Deutsche Forschungsgemeinschaft (SFB 553; HU 842/2-1; JP 244/1-1) and the Stiftung VERUM für Verhalten und Umwelt.

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