Dynamic Cell Biology ©2007 Global Science Books

Enhanced Cell Proliferation Induced by Nitric Oxide

Antonio Villalobo

Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas & Universidad Autónoma de Madrid. c/ Arturo Duperier 4, E-28029 Madrid, Spain

Correspondence: **antonio.villalobo@iib.uam.es**

ABSTRACT

Nitric oxide (NO$^•$) regulates multiple physiological functions including cell proliferation. The regulatory action of NO$^•$ on cell proliferation is exerted in a bimodal mode, enhancing and inhibiting the progression of the proliferative process depending on the actual concentration of NO$^•$ encountered by the cell. Although not all details are understood, the cytostatic action of NO$^•$, when attained at high concentrations, has been studied in more depth. However, the stimulatory action of minute quantities of NO$^•$ on cell proliferation has received less attention, although its potential physiological importance is already apparent. Knowledge on the molecular mechanisms and signalling events responsible for the enhancement of cell proliferation induced by NO$^•$ are still very limited, although new findings have started to uncover some of their details. In this review I shall describe the progress done in the last few years on this respect, and explore the physiological relevance of the enhancement exerted by NO$^•$ on the proliferation of cells relevant for the regulation of normal organismal growth and development. The action of NO$^•$ stimulating the proliferation of tumour cells has became also an important pathophysiological issue and it will be outlined. The studies concerning the action of NO$^•$ on cancerous cells could be significant to better understand the molecular mechanisms underlying this pathological process and its possible therapeutic control.

Keywords: Cell cycle, growth factors, nitric oxide synthase, tumour growth.

Abbreviations: 17β-OestR, 17β-oestrogen receptor; **bFGF**, basic fibroblast growth factor; **$[Ca^{2+}]_{cyt}$**, cytosolic concentration of free Ca^{2+}; **CaM**, calmodulin; **carboxy-PTIO**, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; **CCK-8**, cholestocystokinin-8; **Cdk2**, cyclin-dependent kinase 2; **Cdk4**, cyclin-dependent kinase 4; **cGMP**, cyclic guanosine-monophosphate; **COX-2**, cyclooxygenase-2; **DETA/NO**, 1,1-diethyl-2-hydroxy-2-nitrosohydrazine; **eNOS (NOS-III)**, endothelial NOS (isoform III); **FGFR**, fibroblast growth factor receptor; **GDP**, guanosine-diphosphate; **GSNO**, *S*-nitrosoglutathione; **GTP**, guanosine-triphosphate; **IFNγ**, interferon-γ; **IFNγR**, interferon-γ receptor; **IL-1β**, interleukin-1β; **IL-1βR**, interleukin-1β receptor; **iNOS (NOS-II)**, inducible NOS (isoform II); **IP3R**, inositol-1,4,5-trisphosphate receptor; **L-NAME**, N^{ω}-nitro-L-arginine methyl ester; **L-NIL**, L-*N6*-(1-imino-ethyl)-lysine; **LPS**, lipopolysaccharide; **LPSR**, lipopolysaccharide receptor; **NADPH**, nicotinamide adenosine dinucleotide phosphate (reduced form); **nNOS (NOS-I)**, neuronal NOS (isoform I); **NOS**, NO$^•$ synthase; **NTG**, nitroglycerine; **PI3K**, phosphatidylinositol-3-kinase; **PKG**, protein kinase G; **PLCγ**, phospholipase Cγ; **pRb**, retinoblastoma protein; **ROS**, reactive oxygen species; **SIN-1**, 3-(morpholinosydnonimine); **SNAP**, *S*-nitroso-*N*-acetyl-penicillamine; **SNP**, sodium nitropruside; **TNFα**, tumour necrosis factor-α; **TNFαR**, tumour necrosis factor-α receptor; **VEGF**, vascular endothelial growth factor; **VEGFR**, vascular endothelial growth factor receptor.

CONTENTS

INTRODUCTION ... 60
NO$^•$ as a stimulator of cell proliferation ... 61
Implication of NO$^•$ in tumour cells growth ... 61
Mechanistic insights on NO$^•$-induced proliferation ... 62
FUTURE PERSPECTIVES ... 63
ACKNOWLEDGEMENTS ... 63
REFERENCES ... 63

INTRODUCTION

Nitric oxide (NO$^•$) is a highly reactive free radical gas that is physiologically generated by organisms ranging from bacteria to humans. Its synthesis in mammalian cells during the transformation of the substrate L-arginine to L-citrulline with the concourse of O_2 is catalysed by three isoforms of the enzyme nitric oxide synthase (NOS), using the reducing power of the electron donor NADPH. The NOS family is composed of the so-called neuronal (nNOS; NOS-I), inducible (iNOS; NOS-II) and endothelial (eNOS; NOS-III) isoforms of the enzyme. The constitutive nNOS and eNOS isoforms are regulated by the Ca^{2+}/calmodulin (CaM) complex, which is formed upon rise of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) induced by multiple extracellular stimuli, while CaM is an intrinsic subunit of iNOS. NO$^•$ exerts multiple biological actions working as an intracellular and extracellular messenger encompassing the regulation of the functionality of the vascular system, neurological functions, and the control of some aspects of the immune response, among other emerging roles of great physiological significance.

Not surprisingly, among the new uncovered roles of NO$^•$ the regulation of cell proliferation stands out. As many reports attest, the major effect exerted by NO$^•$ on cell proliferation in many normal and tumour cell types appears to be the arrest of cell cycle progression and the subsequent inhibition of the proliferative process (see for review Villalobo 2006). There are, however, a significant number of reports describing the stimulatory action of NO$^•$ on cell proliferation. The reason for this biphasic phenomenon is not yet well understood in great detail, although recent findings

Received: 25 June, 2006. Accepted: 19 October, 2006.

Invited Mini-Review

suggest that the actual concentration of NO˙ is a critical factor for this dual behaviour. Thus, increasing proliferation at low concentrations (i.e. using NO˙ donors in the µmolar or sub-mmolar ranges in most cases) inhibiting proliferation when its concentration rises (i.e. using NO˙ donors in the mmolar range). In what follows, I shall describe a number of observations related to the stimulatory effect of NO˙ on the proliferation of normal and tumour cells, some of the molecular mechanisms that underlie this process, and the physiological significance of this puzzling phenomena.

NO˙ as a stimulator of cell proliferation

The analysis of the impact of suppression of endogenous NO˙ production and/or the impairment of its availability to the cell could provide a good indication of whether NO˙ has a positive action on cell proliferation, or not. Thus, it has been demonstrated that in lung epithelial and pleural mesothelial cells the depletion of endogenous NO˙ by carboxy-PTIO, an NO˙ scavenger compound, stops cell proliferation by arresting the cell cycle at the late S or G2/M phases, events that are mediated by a cGMP-dependent pathway (Janssen *et al.* 1998).

Moreover, the knockout of the nNOS gene results in decreased proliferation of neural precursors with the subsequent depletion of immature neurones in the olfactory epithelium of new-born mice, and altered organisation of glomerular cell layers in the adult olfactory bulb, suggesting a positive action of NO˙ on early postnatal development (Chen *et al.* 2004). This latter assertion appears to be corroborated in other regions of the central nervous system using a different experimental approach. Thus, it has been demonstrated that proliferation of granule cell precursors in the adult dentate gyrus, induced by generalised clonic seizures after pentylenetrazol treatment, was drastically reduced by an nNOS inhibitor (Jiang *et al.* 2004). NO˙ also enhances cell proliferation in neuroepithelial cells of the developing neural tube (Traister *et al.* 2002; Plachta *et al.* 2003). Intriguingly, in this experimental system high NO˙ concentrations promote the G1 to S phase transition in nuclei that are located in a basal position within the cell, while low NO˙ concentrations promote the G2 to M transition in nuclei with an apical cellular location (Traister *et al.* 2002). Taken together, these reports suggest that NO˙ plays a relevant role in neurogenesis.

It has also been demonstrated that cardiomyocytes isolated from eNOS knockout mice ($eNOS^{-/-}$) proliferate less efficiently than cardiomyocytes from wild type animals, and that cardiac cells proliferate *in vivo* less efficiently in neonatal hearts of $eNOS^{-/-}$ animals (Lepic *et al.* 2006). The implication of NO˙ as a stimulator of cell proliferation in that system was further corroborated by the enhanced proliferation attained by cardiomyocytes from knockout animals upon the addition of DETA/NO, an NO˙ donor, and the suppression of proliferation in cardiomyocytes from wild type animals upon addition of L-NAME, a NOS inhibitor (Lepic *et al.* 2006). Mechanistically, the vascular endothelial growth factor (VEGF) appears to channel its proliferative action in cardiomyocytes via NO˙ as this growth factor lacks an effect in $eNOS^{-/-}$ mice, suggesting that NO˙ plays an important role during postnatal heart development (Lepic *et al.* 2006).

An interesting case is exemplified by embryonic cardiomyocytes, where endogenous NO˙ produced upon tumour necrosis factor-α (TNFα) and lipopolysaccharide (LPS) treatment exerts a stimulatory action on cell proliferation while exogenously added NO˙ has an inhibitory role (Pignatti *et al.* 1999). In contrast, in senescent fibroblasts the induction of iNOS by TNFα, interferon-γ (IFNγ) and interleukin-1β (IL-1β) inhibits cell proliferation, while exogenously supplied NO˙ yields increased cell proliferation and accumulation of cells at the S/G2 phases (Gansauge *et al.* 1997). These paradoxical behaviours could be related to distinct concentrations of NO˙ attained in different experimental conditions within the cellular systems used, as low and high concentrations of exogenous NO˙ enhances and depresses, respectively, the proliferation of fibroblasts (Du *et al.* 1997), pancreatic tumours (Hajri *et al.* 1998), myoblasts (Ulibarri *et al.* 1999), keratinocytes (Krischel *et al.* 1998; Frank *et al.* 2000), vein endothelial cells (Luczak *et al.* 2004), and pheochromocytoma PC12 cells (Bal-Price *et al.* 2006), among others.

The proliferation of kidney glomelular endothelial cells mediated by VEGF is also under the control of NO˙ as the treatment of experimental animals with L-NIL, an iNOS inhibitor, decreases its proliferation, while no effect of the inhibitor was detected in non-endothelial cells in the same tissue preparations (Ostendorf *et al.* 2004). Furthermore, in human umbilical vein endothelial cells the NOS inhibitor L-NAME blocks VEGF-induced proliferation (Hood and Granger 1998).

A supplement of L-arginine increases nNOS expression in retinal cells concomitant with increased proliferation (Kim *et al.* 2002). This suggests that NO˙ may also have a proliferative action in these cells. Nevertheless, reports describing the inhibitory action of NO˙ on retinal cells have been published (Goureau *et al.* 1993; Yilmaz *et al.* 2000), suggesting again that the actual concentration of NO˙ attained in different experimental conditions may be a decisive factor to explain this apparent disagreement.

Proliferation of the exocrine pancreas appears to be regulated also in a bimodal manner by endogenous NO˙. In this case, during cholestocystokinin-8 (CCK-8)-induced hyperplasia, NO˙ induces proliferative arrest in acinar cells but increases proliferation in ductal cells, while enhancing cell proliferation in basal non CCK-8-stimulated conditions (Trulsson *et al.* 2002). Moreover, during CCK-8-induced hypoplasia, exogenous NO˙ enhances acinar cell turnover, suppress apoptosis, and favours cell regeneration increasing both proliferation and cell death via a non-apoptotic pathway (Trulsson *et al.* 2004).

Table 1 summarizes some examples in which NO˙ has been shown to stimulate the proliferation of different normal and tumour cell types *in vitro* upon the addition of NO˙ donors of various chemical structures. The NO˙ donors used in each instance and the concentration range at which enhanced proliferation was observed are indicated.

Implication of NO˙ in tumour cells growth

The action of NO˙ on tumour development and progression, its positive role in the induction of tumour-associated angiogenesis and its dual activating and inhibiting roles in tumour cell proliferation has been studied in detail (see for review Fukumura *et al.* 2006). In this section, therefore, I shall give a few examples on the stimulatory role of NO˙ on the proliferation of different tumour cells.

Exogenous NO˙ enhances the proliferation of human choriocarcinoma JEG-3 cells and prevents the differentiation of cytotrophoblasts to syncytial cells, processes that could be mediated by the expression of nNOS (Sanyal *et al.* 2000). The NO˙-mediated increased proliferation of choriocarcinoma cells is not universal, however, as endogenous and exogenous NO˙ inhibits the proliferation of the trophoblast-like choriocarcinoma BeWo cell line (Cha *et al.* 2001). Ovarian carcinoma cell proliferation is negatively regulated by high concentrations of 17β-oestradiol, and negatively or positively regulated by progesterone depending on its concentration. These effects appear to be controlled by a positive action of NO˙ on cell proliferation as lower growth correlates with decreased iNOS expression and *vice versa* (Bechtel and Bonavida 2001). In human A375 malignant melanoma cells the proliferative response to VEGF appears to be mediated by endogenously produced NO˙ because the enhanced proliferation of cells transfected with VEGF is accompanied by iNOS overexpression, and the NOS inhibitor L-NAME, restrains its proliferation (Tao *et al.* 2005). Moreover, although NO˙ has been implicated in the proliferative arrest of normal T lymphocytes, its implication in the sustained proliferation of the T lymphoma BW5147 cell

Table 1 Stimulatory action of exogenous NO˙ on the proliferation of cultured cell.

Cells	NO˙ donor	Concentration (µM)	Reference
Coronary venule endothelial cells (bovine)	SNP	1-100	Ziche *et al*. 1997
Umbilical vein endothelial cells (human)	SNP	1	Luczak *et al*. 2004
	GSNO	1	
	NTG	0.022	
	SIN-1*	100	
Fetoplacental artery endothelial cells (sheep)	SNP	1	Zheng *et al*. 2006
Cardiomyocytes (mouse)	DETA/NO	2	Lepic *et al*. 2006
Myoblasts (turkey)	SNP	1-10	Ulibarri *et al*. 1999
	SNAP	5-10	
Mesangial MES-13 cells (murine)	SNP	100-200	Sheu *et al*. 2005
	SNAP	100-500	
Keratinocytes (human)	DETA/NO	50-250	Krischel *et al*. 1998
	SNAP	50-500	
HaCaT keratinocytes (human)	GSNO	100	Frank *et al*. 2000
	DETA/NO	100	
MC3T3-E1 osteoblasts (mouse)	SNAP	10-100	Kanamaru *et al*. 2001
BALB/c 3T3 fibroblasts (mouse)	SNAP	5-20	Du *et al*. 1997
	GSNO	25-50	
WI38 fibroblasts** (human)	SNP	1000	Gansauge *et al*. 1997
	SNAP	1000	
Pancreatic tumour HA-hpc2 cells (human)	SNP	250-1000	Hajri *et al*. 1998
Ovarian carcinoma HOC-7 cells (human)	SNAP	10	Bechtel and Bonavida 2001
Choriocarcinoma JEG-3 cells (human)	SNP	5-40	Sanyal *et al*. 2000
Pheochromocytoma PC12 cells (rat)	DETA/NO	25-50	Bal-Price *et al*. 2006

The indicated concentrations of NO˙ donors represent the range at which increased cell proliferation was observed. Higher concentrations of NO˙ donors may inhibit cell proliferation (for review see Villalobo 2006). * SIN-1 is an NO˙ plus $O_2^{\cdot -}$ donor. ** Senescent fibroblasts. See text for additional details and the abbreviations list for the full name of the NO˙ donors used.

line was demonstrated, a process that appears to be mediated by iNOS overexpression in the proliferating but not the arrested tumour cells (Barreiro-Arcos *et al.* 2003).

A few examples of enhanced cell proliferation attained *in vitro* upon addition of a variety of NO˙ donors on tumour cells, and the concentration range at which the effect was observed, are also collected at the end of **Table 1**.

Mechanistic insights on NO˙-induced proliferation

Information on the action of NO˙ activating the cell cycle machinery is rather scanty. Thus, there is a single report in which it has been shown that an ill-defined gaseous mixture of nitrogen oxides, possibly containing NO˙, increases the proliferation of lung fibroblasts, a process mediated by enhanced expression of cyclins A, D1 and E; the cyclin-dependent kinases 2 and 4 (Cdk2 and Cdk4); enhanced phosphorylation of the retinoblastoma protein (pRb), and decreased expression of the cyclin-dependent kinase inhibitors $p27^{Kip1}$ and $p16^{Ink4a}$, while no effect was detected on $p21^{Cip1/Waf1}$ expression (Chen *et al.* 2003). Nevertheless, the actual nature of the agent(s) inducing those effects on the cell cycle remains to be established as the complexity of the gaseous mixture used prevents the identification of NO˙ as the direct intervening agent.

In coronary venular endothelial cells, exogenous and endogenous NO˙ both induce a proliferative response by a mechanism implicating basic fibroblast growth factor (bFGF), as an anti-bFGF antibody blocks NO˙-mediated proliferation (Ziche *et al.* 1997). In addition, the proliferative action of exogenous NO˙, via activation of the mitogen-activated protein kinase (MAPK) pathway, was also demonstrated in foetoplacental artery endothelial cells (Zheng *et al.* 2006), suggesting a positive role of NO˙ in angiogenesis. Moreover, in endothelial cells and cardiomyocytes, VEGF not only upregulates the expression of eNOS but activates this enzyme either by increasing the $[Ca^{2+}]_{cyt}$, and subsequent formation of the Ca^{2+}/CaM complex, by activation of the phosphatidylinositol-3-kinase (PI3K)/Akt pathway and/or by recruitment of heat-shock protein 90 (Hsp90) (Duda *et al.* 2004; Lepic *et al* 2006). VEGF-mediated proliferation of umbilical vein endothelial cells and activation of Raf-1, the first kinase of the MAPK cascade, are NO˙-dependent processes controlled by cGMP and its target protein kinase G (PKG) (Hood and Granger 1998). This was demonstrated because of the formation of a Raf-1/PKG complex detected by immunoprecipitation, and because PKG inhibitors prevent both VEGF-induced proliferation and Raf-1 phosphorylation (Hood and Granger 1998). Consistent with the proliferative action of NO˙ mediated by the activation of the MAPK pathway is also the observation that this agent activates its upstream regulator Ras promoting the GDP/GTP exchange because of the *S*-nitrosylation of its cysteine 118 (Lander *et al.* 1995, 1996).

The proliferation and differentiation of osteoblasts induced by 17β-oestradiol is also mediated by the enhanced expression of eNOS (O'Shaughnessy *et al.* 2000). In agreement with this observation, exogenous NO˙, supplied as an NO˙ donor, also has a proliferative effect on osteoblasts, a process that is independent of cGMP (Kanamaru *et al.* 2001). In addition, osteoblasts proliferation, induced by a pulsed electromagnetic field, was prevented by a NOS inhibitor (Diniz *et al.* 2002). However, 17β-oestradiol has been shown to increase NO˙ production by vascular smooth muscle cells, which is accompanied by decreased proliferation concomitant with decreased c-*fos* expression (Yang *et al.* 2002), suggesting that the mechanism of action of the 17β-oestradiol/NO˙ system is not universal, and could well depend on the actual concentration of NO˙ generated and/or available in the system.

Mechanistically, the NO˙-mediated enhanced proliferation of vein endothelial cells appears to require the secondary production of reactive oxygen species (ROS) because superoxide dismutase and catalase in part suppress the stimulatory effect (Luczak *et al.* 2004). Moreover, the stimulatory effect of NO˙ on aortic endothelial cells was the consequence of eNOS activation by the Ca^{2+}/CaM complex. This complex is formed upon rise of the $[Ca^{2+}]_{cyt}$ induced by activation of CD44v10, a hyaluronan receptor, after interacting with its extracellular ligand, and recruitment and activation of inositol-1,4,5-trisphosphate receptors (IP3R) in the endoplasmic reticulum via the cytoskeleton adaptor ankyrin (Singleton and Bourguignon 2004).

Although an early report suggested that proliferation of mesangial cells was not affected by exogenously added or endogenously produced NO˙ (Mohaupt *et al.* 1994), a more recent report demonstrates that iNOS expression in mesangial kidney cells induced by LPS plus IFNγ treatment re-

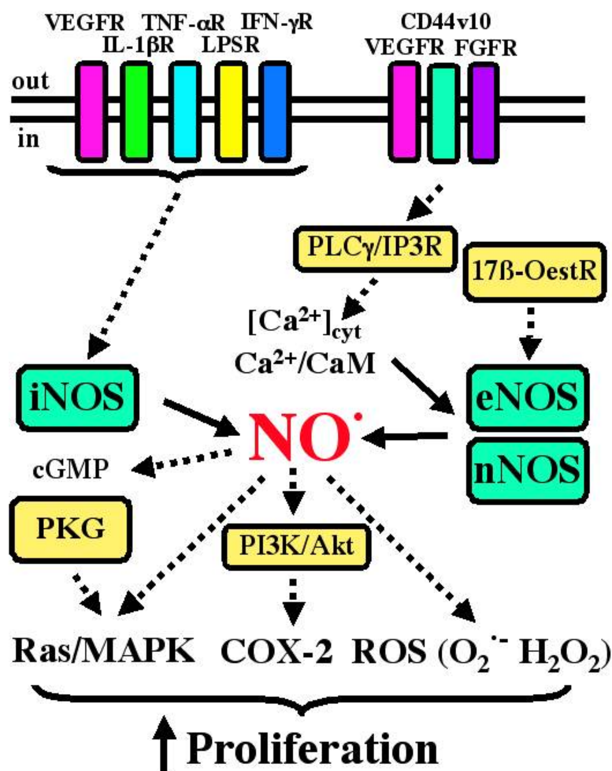

Fig. 1 Extracellular signalling systems responsible for the cellular production of NO•, and some mechanisms implicated in NO•-induced cell proliferation. The activation of a series of plasma membrane-bound receptors, such as vascular endothelial growth factor receptors (VEGFR), tumour necrosis factor-α receptors (TNF-αR), interferon-γ receptors (IFN-γR), interleukin-1β receptors (IL-1βR), and lipopolysaccharide receptors (LPSR) by their cognate extracellular ligands results in the expression of the inducible nitric oxide synthase (iNOS) isoform. On the other hand, the 17β-oestrogen receptor (17β-OestR) controls the expression of the endothelial nitric oxide synthase (eNOS) isoform. Some tyrosine kinase receptors such as VEGFR and the fibroblast growth factor receptor (FGFR), and some non-related receptors such as CD44v10, activated by their extracellular ligands: VEGF, the basic FGF and hyaluronan, respectively, also control NO• production. Activation of the latter plasma membrane-bound receptors induce an increase of the cytosolic concentration of free Ca^{2+} ($[Ca^{2+}]_{cyt}$) because production of inositol-1,4,5-trisphosphate by phospholipase Cγ (PLCγ) and Ca^{2+} released from the endoplasmic reticulum mediated by the subsequent activation of Ca^{2+} channels such as inositol-1,4,5-trisphosphate receptors (IP3R). The ensuing Ca^{2+} rise results in the formation of the Ca^{2+}/calmodulin (Ca^{2+}/CaM) complex and the subsequent activation of eNOS and nNOS. NO• also activates the cytosolic guanylyl cyclase inducing the production of cGMP. This cyclic nucleotide activates protein kinase G (PKG) which phosphorylates some target proteins such as the kinase Raf-1 (not shown) to initiate the activation of the mitogen activated protein kinase (MAPK) pathway. Among the few mechanisms already suggested to be implicated in NO•-mediated stimulation of cell proliferation are: i) the activation of the Ras/MAPK pathway; ii) the upregulation of cyclooxygenase-2 (COX-2) via the phosphatidylinositol-3-kinase (PI3K)/Akt pathway; and iii) the secondary production of reactive oxygen species (ROS) such as anion superoxide ($O_2^{\bullet-}$) and oxygen peroxide (H_2O_2). See text for additional details.

sults in enhanced proliferation because of the NO•-mediated upregulation of cyclooxygenase-2 (COX-2) via the PI3K/Akt pathway (Sheu *et al.* 2005). In contrast, in vascular smooth muscle cells the observed NO•-mediated proliferative arrest appears to be mediated by COX-2, and COX-2-dependent TNFα-induced proliferation appears to be controlled by NO• (Haider *et al.* 2003).

A summary of some known molecular mechanisms and signalling pathways implicated in the cellular production of NO• and the subsequent stimulation of cell proliferation induced by this agent in a prototypic cell is depicted in **Fig. 1**.

FUTURE PERSPECTIVES

The implication of NO• in the control of cell proliferation is an area of great research interest as its study could provide a better understanding of major physiological processes such as tissue remodelling during development and adulthood, and mechanisms of repair after tissue injury. Moreover, the study of the consequences of the dysregulation of NO•-mediated control mechanisms implicated in different pathologies characterized by cell hyperproliferation, such as cancer, could give new insights on the genesis and progression of these pathological processes. However, to better establish the physiological significance of NO• as an inducer of cell proliferation, further studies should be done to clarify the molecular mechanisms of action of this agent, particularly to identify the target molecules and signalling pathways affected during this process, and posttranslational modifications that protein targets suffer upon exposure to NO•, such as *S*-nitrosylation and/or tyrosine nitration, and to determine whether additional mechanisms related to the activation of the soluble guanylyl cyclase and the subsequent increase in cGMP are implicated. Another point of interest in this area of research is to establish the concentration threshold required for NO• *in vivo* to shift its action from being an enhancer of cell proliferation to be an antiproliferative agent, and to determine the cellular mechanisms required to attain this adaptation.

ACKNOWLEDGEMENTS

Research in the author's laboratory was financed by the *Dirección General de Investigación, Ministerio de Educación y Ciencia* (SAF2005-00631). The generous support from the *Fondo de Investigaciones Sanitarias* (RTICCC C03/10) and the European Commission (contract number MRTN-CT-2005-019561) is also acknowledged.

REFERENCES

Bal-Price A, Gartlon J, Brown GC (2006) Nitric oxide stimulates PC12 cell proliferation via cGMP and inhibits at higher concentrations mainly via energy depletion. *Nitric Oxide* **14**, 238-246

Barreiro-Arcos ML, Gorelik G, Klecha A, Goren N, Cerquetti C, Cremaschi GA (2003) Inducible nitric oxide synthase-mediated proliferation of a T lymphoma cell line. *Nitric Oxide* **8**, 111-118

Bechtel MK, Bonavida B (2001) Inhibitory effects of 17β-estradiol and progesterone on ovarian carcinoma cell proliferation: a potential role for inducible nitric oxide synthase. *Gynecologic Oncology* **82**, 127-138

Cha M-S, Lee M-J, Je G-H, Kwak J-Y (2001) Endogenous production of nitric oxide by vascular endothelial growth factor down-regulates proliferation of choriocarcinoma cells. *Biochemical and Biophysical Research Communications* **282**, 1061-1066

Chen J-H, Tseng T-H, Ho Y-C, Lin H-H, Lin W-L, Wang C-J (2003) Gaseous nitrogen oxides stimulate cell cycle progression by retinoblastoma phosphorylation via activation of cyclins/Cdks. *Toxicological Sciences* **76**, 83-90 (erratum in *Toxicological Sciences* **77**, 2004)

Chen J, Tu Y, Moon C, Matarazzo V, Palmer AM, Ronnett GV (2004) The localization of neuronal nitric oxide synthase may influence its role in neuronal precursor proliferation and synaptic maintenance. *Developmental Biology* **269**, 165-182

Diniz P, Soejima K, Ito G (2002) Nitric oxide mediates the effects of pulsed electromagnetic field stimulation on the osteoblast proliferation and differentiation. *Nitric Oxide* **7**, 18-23

Du M, Islam MM, Lin L, Ohmura Y, Moriyama Y, Fujimura S (1997) Promotion of proliferation of murine BALB/c3T3 fibroblasts mediated by nitric oxide at lower concentrations. *Biochemistry and Molecular Biology International* **41**, 625-631

Duda DG, Fukumura D, Jain RK (2004) Role of eNOS in neovascularization: NO for endothelial progenitor cells. *Trends in Molecular Medicine* **10**, 143-145

Frank S, Kämpfer H, Podda M, Kaufmann R, Pfeilschifter J (2000) Identification of copper/zinc superoxide dismutase as a nitric oxide-regulated gene in human (HaCaT) keratinocytes: implications for keratinocyte proliferation. *The Biochemical Journal* **346**, 719-728

Fukumura D, Kashiwagi S, Jain RK (2006) The role of nitric oxide in tumour progression. *Nature Reviews Cancer* **6**, 521-534

Gansauge S, Gansauge F, Nussler AK, Rau B, Poch B, Schoenberg MH, Beger HG (1997) Exogenous, but not endogenous, nitric oxide increases proliferation rates in senescent human fibroblasts. *Federation of European Bio-*

chemical Societies Letters **410**, 160-164

Goureau O, Lepoivre M, Becquet F, Courtois Y (1993) Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor β in bovine retinal pigmented epithelial cells: inverse correlation with cellular proliferation. *Proceedings of the National Academy of Sciences of the USA* **90**, 4276-4280

Haider A, Lee I, Grabarek J, Darzynkiewicz Z Ferreri NR (2003) Dual functionality of cyclooxygenase-2 as a regulator of tumor necrosis factor-mediated G_1 shortening and nitric oxide-mediated inhibition of vascular smooth muscle cell proliferation. *Circulation* **108**, 1015-1021

Hajri A, Metzger E, Vallat F, Coffy S, Flatter E, Evrard S, Marescaux J, Aprahamian M (1998) Role of nitric oxide in pancreatic tumour growth: *in vivo* and *in vitro* studies. *British Journal of Cancer* **78**, 841-849

Hood J, Granger HJ (1998) Protein kinase G mediates vascular endothelial growth factor-induced Raf-1 activation and proliferation in human endothelial cells. *The Journal of Biological Chemistry* **273**, 23504-23508

Janssen YMW, Soultanakis R, Steece K, Heerdt E, Singh RJ, Joseph J, Kalyanaraman B (1998) Depletion of nitric oxide causes cell cycle alterations, apoptosis, and oxidative stress in pulmonary cells. *American Journal of Physiology* **275**, L1100-L1109

Jiang W, Xiao L, Wang J-C, Huang Y-G, Zhang X (2004) Effects of nitric oxide on dentate gyrus cell proliferation after seizures induced by pentylenetetrazol in the adult rat brain. *Neuroscience Letters* **367**, 344-348

Kanamaru Y, Takada T, Saura R, Mizuno K (2001) Effect of nitric oxide on mouse clonal osteogenic cells, MC3T3-E1, proliferation *in vitro*. *Kobe Journal of Medical Sciences* **47**, 1-11

Kim KY, Moon JI, Lee EJ, Lee YJ, Kim IB, Park CK, Oh SJ, Chun MH (2002) The effect of L-arginine, a nitric synthase substrate, on retinal cell proliferation in the postnatal rat. *Developmental Neuroscience* **24**, 313-321

Krischel V, Bruch-Gerharz D, Suschek C, Kröncke K-D, Ruzicka T, Kolb-Bachofen V (1998) Biphasic effect of exogenous nitric oxide on proliferation and differentiation in skin derived keratinocytes but not fibroblasts. *Journal of Investigative Dermatology* **111**, 286-291

Lander HM, Ogiste JS, Pearce SFA, Levi R, Novogrodsky A (1995) Nitric oxide-stimulated guanine nucleotide exchange on $p21^{ras}$. *The Journal of Biological Chemistry* **270**, 7017-7020

Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC, Quilliam LA (1996) Redox regulation of cell signalling. *Nature* **381,** 380-381

Lepic E, Burger D, Lu X, Song W, Feng Q (2006) Lack of endothelial nitric oxide synthase decreases cardiomyocyte proliferation and delays cardiac maturation. *American Journal of Physiology Cell Physiology* **291**, C1240-C1246

Luczak K, Balcerczyk A, Soszynski M, Bartosz G (2004) Low concentration of oxidant and nitric oxide donors stimulate proliferation of human endothelial cells *in vitro. Cell Biology International* **28,** 483-486

Mohaupt M, Schoecklmann HO, Schulze-Lohoff E, Sterzel RB (1994) Altered nitric oxide production and exogenous nitric oxide do not affect the proliferation of rat mesangial cells. *Journal of Hypertension* **12**, 401-408

O'Shaughnessy MC, Polak JM, Afzal F, Hukkanen MVJ, Huang P, MacIntyre I, Buttery LDK (2000) Nitric oxide mediates 17β-estradiol-stimulated human and rodent osteoblast proliferation and differentiation. *Biochemical and Biophysical Research Communications* **277**, 604-610

Ostendorf T, van Roeyen C, Westenfeld R, Gawlik A, Kitahara M, de Heer E, Kerjaschki D, Floege J, Ketteler M (2004) Inducible nitric oxide synthase-derived nitric oxide promotes glomerular angiogenesis *via* upregulation of vascular endothelial growth factor receptors. *Journal of the American Society of Nephrology* **15**, 2307-2319

Plachta N, Traister A, Weil M (2003) Nitric oxide is involved in establishing the balance between cell cycle progression and cell death in the developing neural tube. *Experimental Cell Research* **288**, 354-362

Pignatti C, Tantini B, Stefanelli C, Giordano E, Bonavita F, Clo C, Caldarera CM (1999) Nitric oxide mediates either proliferation or cell death in cardiomyocytes: involvement of polyamines. *Amino Acids* **16**, 181-190

Sanyal M, Nag TC, Das C (2000) Localization of nitric oxide synthase in human trophoblast cells: role of nitric oxide in trophoblast proliferation and differentiation. *American Journal of Reproductive Immunology* **43**, 70-77

Sheu ML, Chao KF, Sung YJ, Lin WW, Lin-Shiau SY, Liu SH (2005) Activation of phosphoinositide 3-kinase in response to inflammation and nitric oxide leads to the up-regulation of cyclooxygenase-2 expression and subsequent cell proliferation in mesangial cells. *Cellular Signalling* **17**, 975-984

Singleton PA, Bourguignon LYW (2004) CD44 interaction with ankyrin and IP3 receptor in lipid rafts promotes hyaluronan-mediated Ca^{2+} signaling leading to nitric oxide production and endothelial cell adhesion and proliferation. *Experimental Cell Research* **295**, 102-118

Tao J, Tu Y-T, Li J-W, Feng A-P, Huang C-Z, Wu Y, Shen G-X (2005) Endogenous production of nitric oxide contributes to proliferation effect of vascular endothelial growth factor-induced malignant melanoma cells. *Clinical and Experimental Dermatology* **31**, 94-99

Traister A, Abashidze S, Gold V, Plachta N, Karchovsky E, Patel K, Weil M (2002) Evidence that nitric oxide regulates cell-cycle progression in the developing chick neuroepithelium. *Developmental Dynamics* **225**, 271-276

Trulsson LM, Gasslander T, Sundqvist T, Svanvik J (2002) The influence of nitric oxide on basal and cholecystokinin-8-induced proliferation and apoptosis in the rat pancreas. *Regulatory Peptides* **106**, 97-104

Trulsson LM, Gasslander T, Svanvik J (2004) Cholecystokinin-8-induced hypoplasia of the rat pancreas: influence of nitric oxide on cell proliferation and programmed cell death. *Basic and Clinical Pharmacology and Toxicology* **95**, 183-190

Ulibarri JA, Mozdziak PE, Schultz E, Cook C, Best TM (1999) Nitric oxide donors, sodium nitroprusside and *S*-nitro-*N*-acetylpencillamine, stimulate myoblast proliferation *in vitro. In vitro Cell Developmental Biology - Animal* **35**, 215-218

Villalobo A (2006) Nitric oxide and cell proliferation. *Federation of European Biochemistry Societies Journal* **273**, 2329-2344

Yang D, Tan Z, Pan JY, Wang TH (2002) 17β-estradiol inhibits vascular smooth muscle cell proliferation and c-*fos* expression: role of nitric oxide. *Sheng Li Xue Bao* **54**, 17-22

Yilmaz G, Esser P, Kociek N, Heimann K (2000) Effect of nitric oxide on proliferation of human retina pigment epithelial cells. *Eye* **14**, 899-902

Zheng J, Wen Y, Austin JL, Chen DB (2006) Exogenous nitric oxide stimulates cell proliferation via activation of a mitogen-activated protein kinase pathway in ovine fetoplacental artery endothelial cells. *Biology of Reproduction* **74**, 375-382

Ziche M, Parenti A, Ledda F, Dell'Era P, Granger HJ, Maggi CA, Presta M (1997) Nitric oxide promotes proliferation and plasminogen activator production by coronary venular endothelium through endogenous bFGF. *Circulation Research* **80**, 845-852