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MINIREVIEW

Inducible NO Synthase Activity in Blood Vessels and Heart: New Insight into Cell Origin and Consequences

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Summary

Induction of the inducible form of nitric oxide synthase (iNOS) in the vascular and cardiac tissue by several inflammatory stimuli may result in the production of large amounts of nitric oxide (NO) for a sustained period. Recent data obtained in the rat aorta in which iNOS was induced by lipopolysaccharide (LPS) have demonstrated that adventitial cells represent the main site of NO production. Adventitial-derived NO can exert an immediate down-regulatory effect on smooth muscle contraction (*via* activation of the cyclic GMP pathway) but may also initiate longer lasting effects through the formation of NO stores within the medial layer. One candidate for such NO stores are dinitrosyl non-heme iron complexes. Low molecular weight thiols interact with preformed NO stores and promote vasorelaxation by a cyclic GMP-independent mechanism involving the activation of potassium channels. In the heart, the induction of iNOS is involved in delayed protection against ischemia-reperfusion-induced functional damages. Recent data obtained with monophosphoryl lipid A, a non-toxin derivative of LPS, strongly suggest that iNOS-derived NO in the rat heart does not act as an immediate mediator of the cardioprotection but rather as a trigger of long-term protective mechanisms. Thus, the present data reveal the important role of adventitial cells as a site of iNOS expression and activity in intact blood vessels. The induction of adaptive mechanisms in the heart and the formation of releasable NO stores in blood vessels are examples of long-term consequences of iNOS induction. These new information are relevant for a better understanding of the circumstances in which NO overproduction by iNOS may play either a beneficial or deleterious role in these tissues.

Key words

Blood vessels • Cardioprotection • Dinitrosyl-iron complexes • Inducible NO synthase • Nitric oxide stores

Introduction

In the vascular and cardiac tissue, nitric oxide (NO) is constitutively produced in modest amount from L-arginine by endothelial (eNOS) or neuronal (nNOS) NO synthase. In response to some cytokines and bacterial products, an inducible form of NOS (iNOS) can be expressed in these tissues. This may result in the

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production of large amounts of NO for sustained periods. In the heart, an increased expression of iNOS has been identified in different types of cardiac failure (such as dilated and inflammatory cardiomyopathy, ischemic heart disease), suggesting its pathophysiological role. However, some experimental data indicate that iNOS may also be involved in cardioprotection (Parratt and Szekeres 1995). In blood vessels, there is no doubt that iNOS expression and activity account for the impaired response to vasoconstrictor agonists that occurs in endotoxemic animals (Stoclet *et al.* 1993). It is generally thought that vascular smooth muscle cells represent the main source of NO in this situation. The activation of soluble guanylyl cyclase and subsequent cyclic GMP accumulation is an important mechanism underlying the effect of NO in heart and blood vessels (Stoclet *et al.* 1998). However, at least from a chemical point of view, the reactivity of NO is broad (Butler *et al.* 1995) and cyclic GMP-independent effects of NO are also expected. These potential multiple pathways may be involved in short- or long-term influences of NO overproduction on vascular or cardiac functions.

This short review will be focused on recent findings obtained in our laboratory, revealing new aspects on the cell origin of iNOS activity, as well as on its short- and long-term consequences in intact vascular and cardiac tissues.

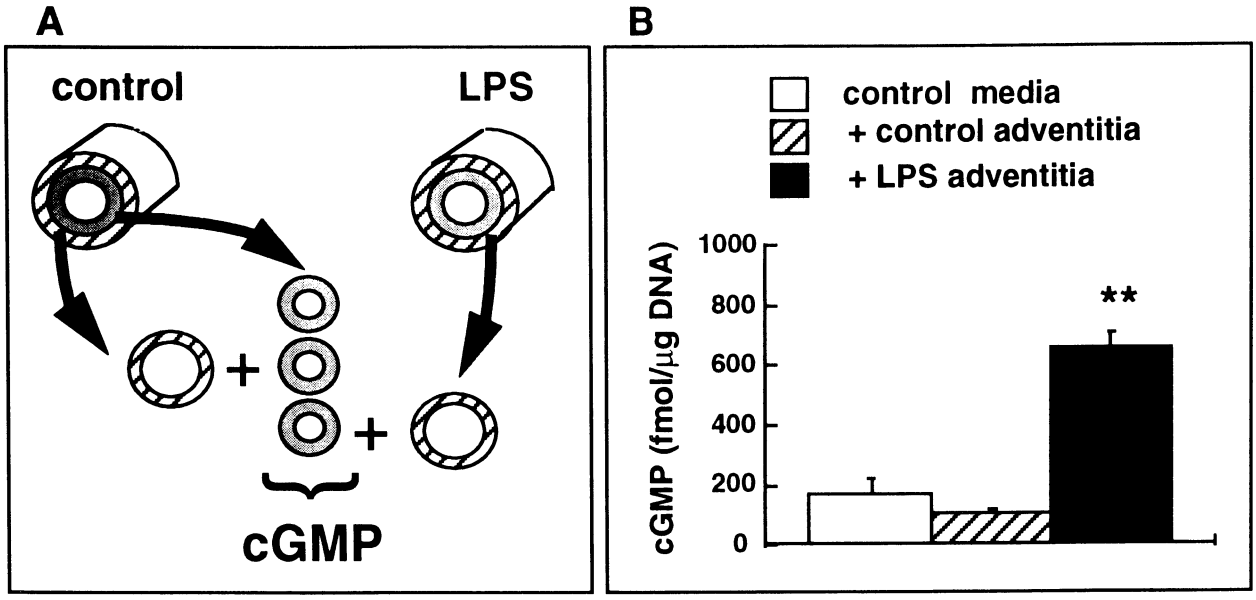

Fig. 1. *[A] Schematic representation of the experimental conditions used for cyclic GMP determination. Medial rings from control aorta were separated and covered or not by adventitial rings from either control aorta or from aorta preincubated for 20 h with lipopolysaccharide (LPS, 10 µg/ml). The preparations were exposed for 30 min to L-arginine (100 µM), isobutylmethylxanthine (0.1 mM) and superoxide dismutase (100 U/ml). The adventitia was removed and cyclic GMP accumulation in the medial layer was determined by radioimmunoassay.* ***[B]*** *Histograms showing the cyclic GMP content in medial rings of control aorta uncovered (open column) or covered by adventitial rings from the control aorta (hatched column) or from LPS-treated aorta (black column).* $^{**}P<0.01$.

Adventitia as a source of NO in LPS-exposed blood vessels

The rat aorta, when exposed for several hours to lipopolysaccharide (LPS) *in vitro* or *in vivo*, displays a decreased response to contractile agonists, profound relaxation upon addition of L-arginine and elevated levels of cyclic GMP. All these modifications are still observed in endothelium-denuded preparations and are reversed by NOS inhibitors (Stoclet *et al.* 1993). It is generally assumed that the main events which affect vascular contraction in LPS-exposed blood vessels (i.e. iNOS expression, NO production and cyclic GMP elevation), mainly take place within the medial layer. The reason for

this is that vascular smooth muscle cells represent the main cell type of the vascular wall and that, in cell culture, they express iNOS after exposure to inflammatory stimuli (Busse and Mülsch 1990). Similarly as smooth muscle cells, fibroblasts and macrophages (the main cell types of the adventitial layer) can also express iNOS in culture in response to inflammatory stimuli. Whether this occurs in intact blood vessels has never been demonstrated.

The expression of iNOS protein was detected in the adventitial layer of rat aorta previously exposed to LPS. Furthermore, iNOS expression was greater in the adventitia than in the media (Kleschyov *et al.* 1997a). The production of NO by the adventitial and medial layer of LPS-treated rat aorta was compared, using classical nitrite plus nitrate (NO_x) determination as well as NO spin trapping followed by electron paramagnetic resonance spectroscopy. In accordance with the distribution iNOS, the adventitia generated much more NO than the media (Kleschyov *et al.* 1997ab, 1998). Experiments on the "reconstructed aorta" were designed to evaluate the ability of adventitial-derived NO to affect soluble guanylyl cyclase activity in the media. Preparations consisted of control media covered by adventitia from control or LPS-treated aorta (Fig. 1). A large elevation of the cyclic GMP content was found in the media previously covered by the adventitia of LPS-treated vessels, but not in the media covered with control adventitia (Fig. 1) (Kleschyov *et al.* 1998). The ability of adventitial-derived NO to affect vascular contraction was further investigated in comparative studies performed on rings with or without adventitia (i.e rings in which the adventitia has been removed or not after LPS-treatment). In contrast to LPS-treated aortic rings with intact adventitia, their medial layers displayed moderate hyporeactivity to noradrenaline and virtually no relaxation upon addition of L-arginine (Kleschyov *et al.* 1998). Altogether, these data demonstrate that not only NO derived from the adventitia can reach the medial layer and influence vascular contraction, but also that the adventitia is potentially the major source of NO in vessels expressing iNOS. These results may be of clinical importance. In septic patients, the administration of L-arginine produces an immediate fall in blood pressure (Lorente *et al.* 1993). As the expression of iNOS was detected in the adventitial layer of small arteries from some septic patients (Stoclet *et al.* 1999a), the hypotensive effect of L-arginine might be due to an increase of adventitial iNOS activity.

NO stores and dinitrosyl-iron complexes in blood vessels

The reversible activation of soluble guanylyl cyclase probably accounts for most of the effect of NO on vascular contraction. However, especially in the case of its overproduction, NO may potentially react with several other targets. For instance, the formation of dinitrosyl-iron complexes (DNIC, $[(RS)_2\ Fe(NO)_2]$) occurs in different cell types or tissues during activation of the iNOS pathway (Henry *et al.* 1993, Vanin and Kleschyov 1998).

Protein-bound DNIC was detected by e.p.r. spectrocopy in aortas incubated with LPS and L-arginine. No characteristic e.p.r. signal for DNIC was found when the aortas were incubated without LPS or L-arginine, or with LPS in the presence of N^{ω}-nitro-L-arginine methyl ester (an inhibitor of NOS activity) or dexamethasone (an inhibitor of iNOS induction) (Muller *et al.* 1996, Kleschyov *et al.* 1997b). These data indicate that protein-bound DNIC were generated *via* LPS-induced iNOS activity. The localization of DNIC was investigated in endothelium-denuded preparations, in which the adventitia was separated from the media after LPS incubation. Under these conditions, the medial layer contained about 4 times more DNIC than the adventitia (3.1±0.5 versus 0.8±0.1 nmol/g wet tissue, respectively) (Kleschyov *et al.* 1997b). The distribution of DNIC in LPS-treated aortas contrasts with that of iNOS activity. Indeed, as mentioned above, NO production was greater in the adventitia than in the media. This suggests a crucial role of adventitia-derived NO in the formation of DNIC within the medial layer. This proposal is further supported by the absence of the e.p.r. signal for DNIC in the media incubated with LPS and L-arginine (Kleschyov *et al.* 1997b).

The role of DNIC is still elusive (Vanin and Kleschyov 1998). In vascular tissue, they may represent NO stores with which low molecular weight thiols may interact to promote relaxation (Mülsch *et al.* 1991). The vasoactive properties of a low molecular weight thiol, N-acetylcysteine (NAC), were characterized in the rat aorta pretreated with LPS. In these preparations, in which ongoing NO production by iNOS was blocked, NAC produced a transient relaxing effect (Fig. 2) (Muller *et al.* 1996). The relaxant effect of NAC in vessels exposed to LPS is related to an interaction with preformed NO stores, since it was not observed when rings were preincubated with LPS in the absence of L-arginine, or in the presence of L-arginine and L-NAME (Fig. 2) (Muller

et al. 1996). NAC-sensitive NO stores were localized in the medial layer, since NAC still produced relaxation of aortic rings after endothelium or adventitia removal. Surprisingly, the relaxation elicited by NAC was neither associated with an elevation of cyclic GMP content, nor affected by inhibition of cyclic GMP-dependent protein kinase (Muller *et al.* 1998a). However, it was attenuated by a potassium channels blocker (tetrabutylammonium) or by the use of elevated concentrations of KCl as the contracting agent (Muller *et al.* 1998a). Thus, NAC-induced relaxation, although being related to preformed NO stores, was mediated by activation of potassium channels rather than by the activation of the cyclic GMP pathway. Interestingly, the activation of some potassium conductance in blood vessels and their subsequent relaxation may result from NO-mediated *S*-nitrosation of critical thiol groups of the potassium channels (Bolotina *et al.* 1994). The precise mechanism of such *S*-nitrosative reactions remains unclear. With regard to the aboved mentioned data and those reported by others (Stamler 1994), low molecular weight thiol may in some way facilitate these *S*-nitrosative reactions.

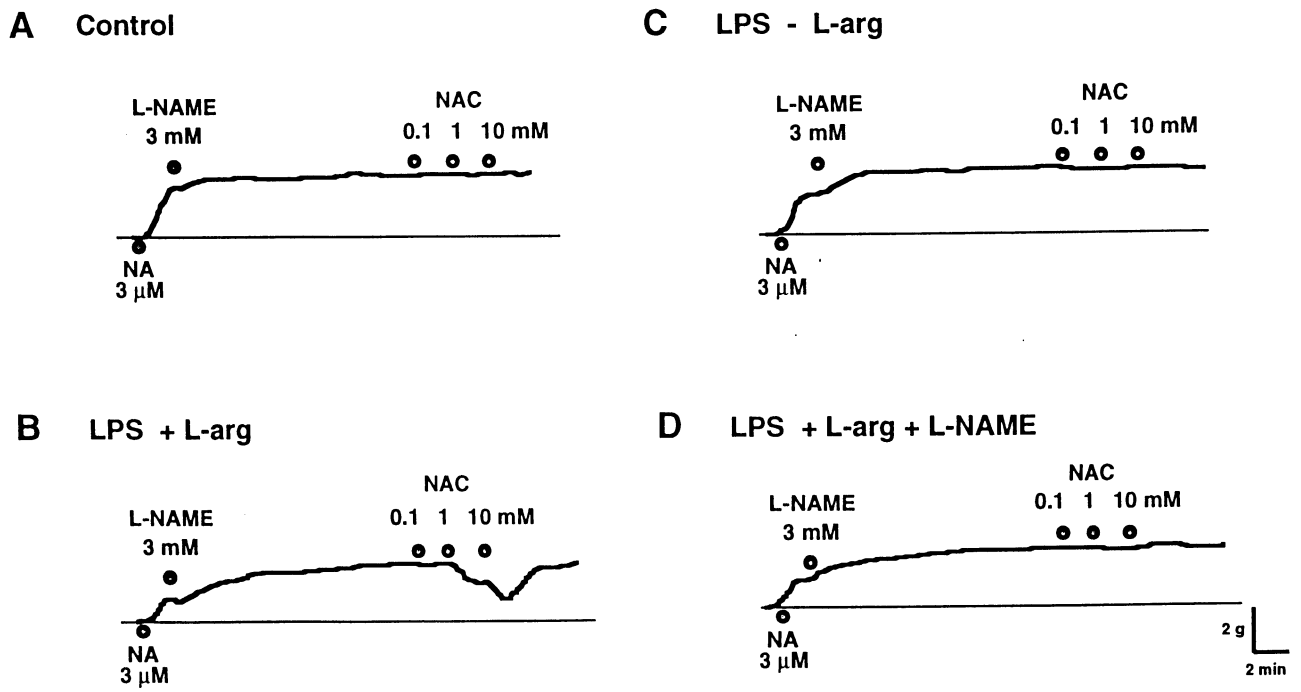

Fig. 2. *Representative traces showing the effect of N-acetylcysteine (NAC) on rat aortic rings incubated for 24 h [A] in the absence or [B] in the presence of lipopolysaccharide (LPS 10 µg/ml) + L-arginine (L-arg, 1 mM), [C] with LPS alone in the absence of L-arg or [D] with LPS + L-arg + N^{ω}-nitro-L-arginine methyl ester (L-NAME, 1 mM). The rings were precontracted with 3 µM noradrenaline (NA) plus 3 mM L-NAME.*

The association between the appearance of DNIC and the relaxing effect of NAC suggests that DNIC may represent NAC-sensitive NO stores in LPS-treated aorta. Some other experimental data support this hypothesis. In LPS-treated aorta, e.p.r. evidence was obtained that NAC (at high concentrations) partially converted protein-bound DNIC into low molecular weight DNIC (Muller *et al.* 1996). Furthermore, the metal chelator diethyldithiocarbamate both destroyed DNIC and prevented the relaxing effect of NAC in these preparations. In addition, aortic rings when pre-exposed to exogenous DNIC exhibited an e.p.r. signal for protein-bound DNIC and relaxation upon addition of NAC (Muller *et al.* 1998b). However, pre-exposure to *S*-nitrosoglutathione (a S-nitrosothiol) also resulted in NAC-induced vasorelaxation (Muller *et al.* 1998b). These data suggest that in blood vessels, especially during large and long-lasting NO production, there may exist other potential forms of NO stores than DNIC, with which low molecular weight thiols may interact to elicit relaxation. Conversely, the role of DNIC may not be confined to NO stores. DNIC might contribute to the cytotoxic action of NO through the inhibition of some key mitochondrial enzymes of the respiratory chain (Henry *et al.* 1993), and

have also been reported to exert antioxidant properties (Gorbunov *et al.* 1997). In addition to releasing NO, DNIC can also transfer their $Fe(NO)_2$ groups to other proteins and, at the same time, are potent *S*-nitrosating agents (Stoclet *et al.* 1998, 1999b). These reactions may be important in the distinct signaling pathways which may be triggered by NO.

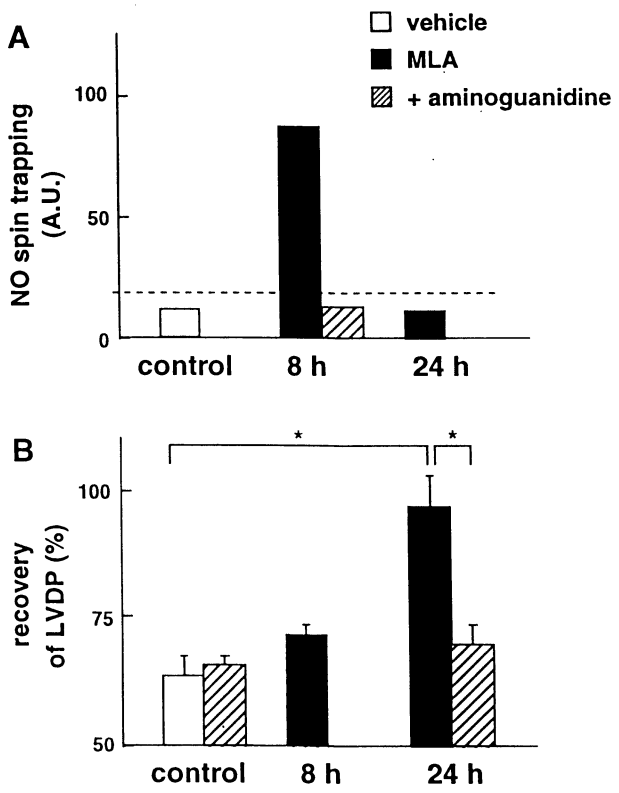

Fig. 3. *Histograms showing [A] NO production and* ***[B]*** *the recovery of left ventricular developed pressure (LVDP) after an episode of global ischemic insult (30 min) followed by 30 min reperfusion, performed in isolated-perfused heart. Hearts were removed from vehicle-treated rats (control), or rats which had received an injection of monophosphoryl lipid A alone 8 h or 24 h before (MLA, 2.5 mg/kg, i.p, black columns) or with MLA + aminoguanidine (2 x 300 mg/kg, hatched columns). Cardiac NO production is expressed in arbitrary units (A.U.). It was determined by e.p.r. spectroscopy in parallel groups of animals, pretreated 30 min before heart removal by the NO spin trapping agent Fe-diethyldithiocarbamate (DETC, 500 mg* kg^{-1}*; i.p. and* $FeSO_4.7H_2O$ *plus Na-citrate, 50 and 250 mg* kg^{-1} *respectively; s.c.). Dotted line indicates limit of detection. Recovery of LVDP is expressed as percentage of preischemic values.* $*p<0.05$.

Triggering role of iNOS-derived NO in cardioprotection

The consequences of iNOS induction in the heart are controversial (for recent review see Stoclet *et al.* 1999). NO derived from iNOS has been involved in delayed cardioprotection, which can result from ischemic preconditioning, heat stress, cardiac pacing or from the administration of exogenous compounds (Parratt and Szekeres 1995). For instance, endotoxin in sublethal doses is effective in reducing the major consequences of coronary artery occlusion, i.e. arrhythmia severity and the extent of myocardial necrosis (Brown *et al.* 1989, Song *et al.* 1996). Much interest has been focused recently on monophosphoryl lipid A (MLA, a synthetic derivative of endotoxin obtained by modification of the lipid A domain of *Salmonella minnesota* LPS). Administered in a single dose 24 h prior to a cardiac ischemic insult, MLA reduces reperfusion damages in various species (Elliott 1998). In the rat, for instance, MLA-pretreatment increased the recovery of left ventricular developed pressure (Fig. 3) and also decreased the incidence of ventricular fibrillations after an episode of ischemia-reperfusion. A role of iNOS in the cardioprotective effect of MLA was demonstrated using iNOS inhibitors in rats (Tosaki *et al.* 1998, György *et al.* 1999) and very recently in iNOS knock-out mice (Xi *et al.* 1999). In the rat heart, the production of NO was evaluated using *in vivo* spin trapping and e.p.r. spectroscopy (György *et al.* 1999). Surprisingly, 24 h after MLA administration (when the protection was evident), no e.p.r. detectable NO was observed in cardiac tissue (Fig. 3). At this time, treatment of isolated hearts removed from MLA-treated animals with aminoguanidine just before the ischemic insult did not attenuate cardioprotection (György *et al.* 1999). Eight hours after MLA administration, however, NO production was increased in the heart, whereas no protection was evident (Fig. 3). Since pretreatment of the rats with iNOS inhibitors (aminoguanidine, but also L-N^6-(1-iminoethyl)-lysine)) abolished MLA-induced NO elevation and cardioprotection, iNOS-derived NO appears to act as a trigger rather than an immediate mediator of the protective effect of MLA (György *et al.* 1999). The induction of antioxidant enzymes is a potential mechanism by which NO derived from iNOS may act as protection against ischemia-reperfusion damages. Indeed, MLA- or endotoxin-induced cardioprotection is associated with increased catalase expression and activity (Brown *et al.* 1989, Nelson *et al.* 1991). Furthermore, NO may upregulate the expression

of superoxide dismutases (Sano *et al.* 1996, Frank *et al.* 1999). The expression of heat shock proteins (HSP), especially the HSP70 family, is another potential mechanism involved in delayed cardioprotection (Richard *et al.* 1996). The ability of NO and MLA to trigger HSP expression has been demonstrated in the heart and in cardiac cells, respectively (Malyshev *et al.* 1996, Nayeem *et al.* 1997). Interestingly, DNIC induces HSP70 accumulation and exerts a delayed protective effect against ischemia-reperfusion injuries in the heart. It has been postulated that DNIC induces *S*-nitrosation of thiols groups of the HSP70 transcription factor, thereby activating the transcription of HSP70 genes (Malyshev *et al* 1996).

Conclusions

High NO production by iNOS occurs during systemic or local inflammatory reactions in the vascular and cardiac tissue. In blood vessels expressing iNOS in response to LPS, adventitial NO production affects smooth muscle contraction by an immediate vasodilatory influence (due to current iNOS activity) and through the generation of NO stores in the media. NO stores may not only provide long-term relaxation, or buffer high NO level, but probably also exert their own effects, as exemplified by the multiple properties of DNIC. The idea that NO produced by iNOS only exerts deleterious effects in the vascular and cardiac tissue is probably oversimplified. As has recently been proposed in the heart, iNOS-derived NO likely plays a role in the induction of cytoprotective mechanisms against oxidative stress. It thus appears that not only NO concentrations but also the site of their production and the local redox state may determine the molecular targets of NO. These interactions may result in short- or long-term biological effects, and probably determine the beneficial or detrimental consequences of NO produced by iNOS in the vascular and cardiac tissue.

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References

BOLOTINA VM, NAJIBI S, PALACINO JJ, PAGANO PJ, COHEN RA: Nitric oxide directly activated calcium-dependent potassium channels in vascular smooth muscle. *Nature* **368**: 850-853, 1994.

BROWN JM, GROSSO MA, TERADA LS, WHITMAN GJR, BANERJEE A, WHITE CW, HARKEN AH, REPINE JE: Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischaemia-reperfusion injury of isolated rat hearts. *Proc Natl Acad Sci USA* **86**: 2516-2520, 1989.

BUSSE R, MÜLSCH A: Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells. *FEBS Lett* **275**: 87-90, 1990.

BUTLER R., FLITNEY FW, WILLIAMS DLH: NO, nitrosonium ions, nitroxide ions, nitrosothiols and iron-nitrosyls in biology: a chemist's perspective. *Trends Pharmacol Sci* **16**: 18-22, 1995.

ELLIOTT GT: Monophosphoryl lipid A induces delayed preconditioning against cardiac ischaemia-reperfusion injury *J Mol Cell Cardiol* **30**: 3-17, 1998.

FRANK S, ZACHAROWSKI K, WRAY GM, THIEMERMANN C, PFEILSCHIFTER J: Identification of copper/zinc superoxide dismutase as a novel nitric oxide-regulated gene in rat glomerular mesangial cells and kidneys of endotoxemic rats. *FASEB J* **13**: 869-882, 1999.

GORBUNOV NV, YALOWICH JC, GADDAM A, THAMPATTY P, RITOV VB, KISIN ER, ELSAYED NM, KAGAN VE: Nitric oxide prevents oxidative damage produced by *tert*-butylhydroperoxyde in erythroleukemia cells via nitrosylation of heme and non heme iron. *J Biol Chem* **272**: 12328-12341, 1997.

GYORGY K, MULLER B, VEGH A, KLESCHYOV AL, STOCLET JC: Triggering role of nitric oxide in the delayed protective effect of monophosphoryl lipid A in rat heart. *Br J Pharmacol,* **127**: 1892-1898, 1999.

HENRY Y, LEPOIVRE M, DRAPIER JC, DUCROCQ C, BOUCHER JL, GUISSANI A: EPR characterization of molecular targets for NO in mammalian cells and organelles. *FASEB J* 7: 1124-1134, 1993.

KLESCHYOV AL, MULLER B, KÉRAVIS T, STOCLET JC: Adventitia-derived nitric oxide triggered by lipopolysaccharide in rat aorta. *J Vasc Res* **34** (suppl 1): 75, 1997a.

KLESCHYOV AL, MULLER B, STOCLET JC: Nitric oxide (NO) store as dinitrosyl-iron complexes in lipopolysaccharide-treated isolated rat aorta: localization and mechanism of formation. *Br J Pharmacol* **120**: 90P, 1997b.

KLESCHYOV AL, MULLER B, SCHOTT C, STOCLET JC: Role of adventitia-derived nitric oxide in vascular hyporeactivity induced by lipopolysaccharide in rat aorta. *Br J Pharmacol* **124**: 623-626, 1998.

LORENTE JA, LANDIN L, DEPABLO R, RENES E, LISTE D: L-arginine pathway in the sepsis syndrom. *Crit Care Med* **21**: 1287-1295, 1993.

MALYSHEV IY, MALUGIN AV, MANUKHINA EB, LARIONOV NP, MALENYUK EB, MALYSHEVA EV, MIKOYAN VD, VANIN AF: Is HSP70 involved in nitric oxide-induced protection of the heart ? *Physiol Res* **45**: 267-272, 1996.

MULLER B, KLESCHYOV AL, STOCLET JC: Evidence for N-acetylcysteine-sensitive nitric oxide stores as dinitrosyl-iron complexes in lipopolysaccharide-treated rat aorta. *Br J Pharmacol* **119**: 1281-1285, 1996.

MULLER B, KLESCHYOV AL, MALBLANC S, STOCLET JC: Nitric oxide-related cyclic GMP-independent effect of N-acetylcysteine in lipopolysaccharide treated rat aorta. *Br J Pharmacol* **123**: 1221-1229, 1998a.

MULLER B, KLESCHYOV AL, MALBLANC S, VANIN A, STOCLET JC: Formation of nitric oxide stores in vascular tissue. *Fundam Clin Pharmacol* **12**: 351, 1998b.

MULLER B, KLESCHYOV AL, STOCLET JC: Inducible NO synthase activity in blood vessels: role of the adventitia and formation of NO stores. *Physiol Res* **48:** 35P, 1999.

MÜLSCH A, MORDVINTCEV P, VANIN AF, BUSSE R: The potent and guanylyl cyclase activating dinitrosyl-iron (II) complex is stored in a protein-bound form in vascular tissue and is released by thiols. *FEBS Lett* **294**: 252-256, 1991.

NAYEEM MA, ELLIOTT GT, SHAH MR, HASTILLO-HESS SL, KUKREJA RC: Monophosphoryl lipid A protects adult rat cardiomyocytes with specific induction of the 72-kD heat shock protein: a cellular model of pharmacological preconditioning. *J Mol Cell Cardiol* **29**: 2305-2310, 1997.

NELSON DW, BROWN JM, BANERJEE A, BENSARD DD, ROGERS KB, LOCKE-WINTER CR, ANDERSON BO, HARKEN AH: Pretreatment with a nontoxic derivative of endotoxin induces functional protection against cardiac ischemia/reperfusion injury *Surgery* **110**: 365-369, 1991.

PARRATT JR, SZEKERES L: Delayed protection of the heart against ischaemia. *Trends Pharmacol Sci* **16**: 351-355, 1995.

RICHARD V, KAEFFER N, THUILLEZ C: Delayed protection of the ischaemic heart – from pathophysiology to therapeutic applications. *Fundam Clin Pharmacol* **10**: 409-415, 1996.

SANO H, HIRAI M, SAITO H, NAKASHIMA I, ISOBE K: Nitric oxide releasing reagent, S-nitroso-N-acetylpenicillamine, enhances the expression of manganese superoxide dismutase mRNA in rat vascular smooth muscle cells. *J Cell Biochem* **62**: 50-55, 1996.

SONG W, FURMAN BL, PARRATT JR: Delayed protection against ischaemia-induced ventricular arrhythmias and infarct size limitation by the prior administration of *Escherichia coli* endotoxin. *Br J Pharmacol* **118**: 2157-2163, 1996.

STAMLER JS: Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* **78**: 931-936, 1994.

STOCLET JC, FLEMING I, GRAY GA, JULOU-SCHAEFFER G, SCHNEIDER F, SCHOTT C, PARRATT JR: Nitric oxide and endotoxemia. *Circulation* **87** (Suppl V)**:** V77-V80, 1993.

STOCLET JC, TRONCY E, MULLER B, BRUA C, KLESCHYOV AL: Molecular mechanisms underlying the role of nitric oxide in the cardiovascular system. *Expert Opin Invest Drugs* **7**: 1769-1779, 1998.

STOCLET JC, MARTINEZ MC, OHLMANN P, CHASSEROT S, SCHOTT C, KLESCHYOV AL, SCHNEIDER F, ANDRIANTSITOHAINA R: Induction of nitric oxide synthase and dual effects of nitric oxide and cyclooxygenase products in regulation of arterial contraction in human septic shock. *Circulation* **100**: 107-112, 1999a.

STOCLET JC, MULLER B, GYORGY K, ANDRIANTSITOHAINA R, KLESCHYOV A: The inducible NO-synthase in vascular and cardiac tissue. *Eur J Pharmacol* **375**: 139-155, 1999b.

TOSAKI A, MAULIK N, ELLIOTT GT, BLASIG IE, ENGELMAN RM, DAS DK: Preconditioning of rat heart with monophosphoryl lipid A: a role for nitric oxide. *J Pharmacol Exp Ther* **285**: 1274-1279, 1998.

VANIN AF, KLESCHYOV AL EPR detection and biological implication of nitrosyl non-heme iron complexes. In: *Nitric Oxide in Transplant Rejection and Antitumor Defense*, SJ LUKIEWICZ, JL ZWEIER (eds), Kluwer Academic Publishers, Norwell, MA, 1998, pp 49-82.

XI L, JARRETT NC, HESS ML, KUKREJA RC: Essential role of inducible nitric oxide synthase in monophosphoryl lipid A induced late cardioprotection: evidence from pharmacological inhibition and gene knockout mice. *Circulation* **99**: 2157-2163, 1999.

Reprint requests

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