Br. J. Pharmacol. (1992), **105**, 575–580 © Macmillan Press Ltd, 1992

Induction and potential biological relevance of a Ca^{2+}-independent nitric oxide synthase in the myocardium

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1 We have investigated whether the myocardium and isolated cardiac myocytes can express a Ca^{2+}-independent NO synthase after treatment with endotoxin or cytokines. Nitric oxide synthesis was measured in cytosols from the left ventricular wall from rats treated with endotoxin, or from freshly isolated myocytes from adult rats treated *in vitro* with cytokines.

2 Cytosols from the ventricle of saline-treated control animals showed only Ca^{2+}-dependent NO synthesis. After treatment with endotoxin, the expression of an inducible, Ca^{2+}-independent NO synthase was observed. The activity of this enzyme was maximal at 6 h and returned towards control levels by 18 h; no alterations occurred in the Ca^{2+}-dependent NO synthase activity. Parallel to this enzyme induction there was an increase in myocardial guanosine 3′:5′-cyclic monophosphate (cyclic GMP) and plasma nitrite and nitrate (NO_x^-). All these changes were prevented by pretreatment of the rats with dexamethasone.

3 Myocytes possessed Ca^{2+}-dependent NO synthase activity and expressed, after treatment with tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), a Ca^{2+}-independent NO synthase, the induction of which was prevented by dexamethasone and cycloheximide.

4 Since increases in cyclic GMP levels in the heart are associated with reduced myocardial contractility, it is possible that the enhanced production of NO by a Ca^{2+}-independent enzyme accounts, at least in part, for the depression of myocardial contractility seen in septic shock, cardiomyopathies, allograft rejection, burn trauma, as well as during anti-tumour therapy with cytokines.

Keywords: Endotoxin; tumour necrosis factor; interleukin-1β; myocytes; heart; nitric oxide synthase; cyclic GMP; nitrites; nitrates; dexamethasone

Introduction

In the vasculature, the basal release of nitric oxide (NO) by a constitutive, Ca^{2+}-dependent NO synthase plays a role in the control of blood pressure and regional blood flow by elevating the levels of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (see Moncada *et al.*, 1991a for review). Treatment with endotoxin or cytokines induces a Ca^{2+}-independent NO synthase in the endothelium (Radomski *et al.*, 1990) and vascular smooth muscle (Knowles *et al.*, 1990a; Rees *et al.*, 1990a; Busse & Mülsch, 1990). The expression of this enzyme leads to vasodilatation and hyporesponsiveness to vasoconstrictors (Rees *et al.*, 1990a; Smith *et al.*, 1991a) which explain the haemodynamic changes in endotoxin shock (Moncada *et al.*, 1991a). These changes, as well as the accompanying progressive increase in the synthesis of NO and cyclic GMP, are inhibited by N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of both Ca^{2+}-dependent and Ca^{2+}-independent NO synthases and by dexamethasone, which prevents the induction of the latter enzyme (see Moncada *et al.*, 1991a, for review).

Although delayed hypotension is a hallmark of septic shock, many studies have revealed that depression in myocardial contractility is a determinant pathophysiological event in the outcome of this condition (Solis & Downing, 1966; Kadowitz & Yard, 1970; Gunteroth *et al.*, 1982; Parker & Adams, 1985; Shepherd *et al.*, 1986; Suffredini *et al.*, 1989). Endotoxin does not have a direct effect on cardiac muscle but acts through the release of products (Abel, 1990), the nature of which and whether they are locally or systemically active have not been established. A myocardial depressant factor (Lefer & Martin, 1970), probably a protein, has been partially isolated (Parrillo *et al.*, 1985; Hallström *et al.*, 1991). However, more recently, cytokines which are released in response to endotoxin challenge (Beutler *et al.*, 1985) have been implicated in myocardial depression (Hollenberg *et al.*, 1989; Hosenpud *et al.*, 1989; Sobotka *et al.*, 1990; Wiechmann *et al.*, 1991).

Increases in cyclic GMP or treatment with its membrane-permeable analogue, 8-bromo-cyclic GMP, have been shown to decrease myocardial contractility (Smith *et al.*, 1991b; Shah *et al.*, 1991; Fort & Lewis, 1991). Furthermore, we have demonstrated in cultured endocardial cells the release of NO by a Ca^{2+}-dependent enzyme (Schulz *et al.*, 1991) which plays a role in the regulation of myocardial contractility (Smith *et al.*, 1991b).

In view of these findings and the possibility that the pathological release of NO might be involved in myocardial dysfunction, we have now investigated whether myocardial tissue *in vivo* and freshly isolated myocytes express the Ca^{2+}-independent, inducible NO synthase when treated with endotoxin or cytokines.

Some of these results were presented at the joint meeting of the British and Nordic Pharmacological Societies, Southampton, 18–20th September, 1991.

Methods

Treatment of rats and preparation of soluble tissue extracts

Male Wistar rats (250–300 g, Charles River) were injected intraperitoneally with: (a) endotoxin (4 mg kg^{-1}), (b) dexamethasone (1 mg kg^{-1}), (c) dexamethasone (1 mg kg^{-1}) followed 30 min later by endotoxin (4 mg kg^{-1}) or (d) with an equivalent volume of pyrogen-free saline. Animals were killed by cervical dislocation after 6 h or at the times indicated. The thoracic cavity was opened and a blood sample was taken immediately by cardiac puncture with a heparinized syringe. The blood sample was centrifuged (10,000 *g*, 1 min) and stored at −20°C for later determination of plasma NO_x^-. The heart was removed, rinsed and placed into cold Krebs buffer gassed with 5% CO_2 in O_2. A 3–4 mm size segment of myocardium

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from the left ventricular wall was excized, avoiding any major blood vessels and the endocardial surface. This was cut into segments, washed thoroughly with three exchanges of Krebs buffer and blotted. One piece (ca. 1–1.5 mm) was frozen separately in liquid nitrogen for determination of cyclic GMP content and the rest was freeze-clamped for determination of ventricular NO synthase activity. The freeze-clamped segments were homogenized in 0.5 ml of ice-cold buffer containing 320 mM sucrose, 10 mM HEPES, 0.1 mM EDTA, 1 mM DL-dithiothreitol, 100 $\mu g\,ml^{-1}$ phenylmethylsulphonyl fluoride, 10 $\mu g\,ml^{-1}$ leupeptin, 10 $\mu g\,ml^{-1}$ soybean trypsin inhibitor, and 2 $\mu g\,ml^{-1}$ aprotinin (adjusted to pH 7.2 at 20°C with HCl) in a Ystral homogenizer. The homogenate was centrifuged (100,000 g, 30 min) at 4°C and the cytosolic (supernatant) fraction was kept on ice for immediate assay of NO synthase activity.

Induction of NO synthase in cardiac myocytes

Cardiac myocytes from adult rats (250–350 g) were isolated by perfusion of the isolated heart with collagenase as described (Powell *et al.*, 1980; Stone *et al.*, 1989). The final cell preparation containing approximately 55% viable (i.e. trypan-blue-excluding, rod-shaped) cells was resuspended (approx. 30,000 rods ml^{-1}) in Dulbecco's MEM supplemented with 10% foetal calf serum, 2 mM glutamine, penicillin (100 u ml^{-1}) and streptomycin (100 $\mu g\,ml^{-1}$). Polymyxin B (10 $\mu g\,ml^{-1}$) was added to bind any endotoxin which may have contaminated the preparation during the myocyte isolation procedure. Cells were incubated for 24 h at 37°C in an atmosphere containing 5% CO_2 in the presence of (a) culture medium alone or (b) TNF-α (20 ng ml^{-1}) and IL-1β (5 ng ml^{-1}), plus (c) cycloheximide (10 μM) or (d) dexamethasone (3 μM) which was added 30 min before the cytokines. Cells were harvested by centrifugation (40 g, 2 min) and the cell pellet washed 3 times with phosphate-buffered saline (PBS). Cell viability was reduced to approx. 38% after 24 h incubations. A final spin (10,000 g, 15 s) was performed to compact the pellet which was resuspended in ice-cold homogenization buffer and sonicated twice for 3 s at 100 W. The cytosolic fraction was prepared as indicated above.

Assay of Ca^{2+}-dependent and Ca^{2+}-independent NO synthase

Nitric oxide formation in the cytosolic fractions of rat ventricular wall and isolated myocytes was measured by the formation of radiolabelled [^{14}C]-citrulline from [^{14}C]-L-arginine, essentially as described by Knowles *et al.* (1990b). Duplicate incubations for 10 min at 37°C were performed for each sample in the presence or absence of either EGTA (1 mM) or EGTA plus L-NMMA (1 mM each) to determine the level of the Ca^{2+}-dependent and Ca^{2+}-independent NO synthase activities, respectively, before terminating the reaction by addition of 0.1 vol of 20% (vol:vol) aqueous $HClO_4$. Samples were neutralized by addition of 0·23 vol of 1.9 M aqueous $KHCO_3$, cooled on ice for 5 min and centrifuged (10,000 g, 2 min). [^{14}C]-citrulline in the supernatant was separated from [^{14}C]-arginine by cation-exchange chromatography using AG 50W-X8 resin and quantified by liquid-scintillation counting.

Determination of cyclic GMP in left ventricular wall

Frozen sements of left ventricular wall were pulverized in a stainless steel pestle and mortar cooled in dry ice. Ice-cold 0.2 M perchloric acid in 80 mM HEPES (0.5 ml) was added. After mixing, homogenates were kept on ice for 10 min and centrifuged (10,000 g, 2 min). The supernatant was removed and neutralized with 1.1 M K_3PO_4 and centrifuged as described above; cyclic GMP levels were determined in duplicate, by specific radioimmunoassay (Moncada *et al.*, 1991b). The protein pellet was solubilized by boiling for 10 min in 2 M NaOH for protein determination.

Determination of plasma NO_x^-

Plasma samples were deproteinized by ultrafiltration (Centrifree micropartition system, Amicon). The nitrate content of the samples was reduced to nitrite with a nitrate reductase (Schmidt *et al.*, 1990). Nitrate reductase (20 mu), phosphate buffer (1.2 M, pH 7.5), FAD (120 mM) and NADPH (14.4 mM) were added to 100 μl of deproteinized plasma or appropriate dilutions in distilled water to give a final volume of 120 μl and incubated for 1 h at 37°C. 5-10 μl of the reduced sample was injected into a reaction vessel containing refluxing 6% aqueous sodium iodide/glacial acetic acid (1:5, vol/vol). Under these conditions, nitrite is reduced to NO and is removed in the gas phase by a constant stream of nitrogen. This was mixed with ozone to form a chemiluminescent product which was then measured in a detector coupled to an electronic integrator (see Palmer *et al.*, 1987). The area under the peak of the chemiluminescence signal is proportional to the amount of nitrite in the sample, as calibrated using a standard solution of $NaNO_2$ and is linear in the range of 5–100 μM. A standard curve of reduction of nitrate to nitrite was performed and used in each experiment as a reference. As plasma contains both nitrite and nitrate the concentrations are reported as total NO_x^- content.

Determination of protein

The protein content of the solubilized protein pellet from ventricular wall extracts for cyclic GMP determination or from the cytosols prepared from homogenates of ventricular wall or myocytes was measured with BCA protein reagent; bovine serum albumin was used as a standard.

Materials

Phenol-extracted lipopolysaccharide (LPS, endotoxin) from *Salmonella typhosa 0901* (Difco), L-[U-^{14}C]-arginine monohydrochloride (305 mCi $mmol^{-1}$), [8-^{3}H]-guanosine 3':5'-cyclic monophosphate (cyclic GMP, 18.2 Ci $mmol^{-1}$), human recombinant IL-1β and TNF-α (Amersham), dexamethasone sodium phosphate (Decadron, Merck Sharpe and Dohme), AG 50W-X8 cation exchange resin (200–400 mesh, Bio-Rad), bicinchoninic acid protein assay reagent (Pierce), NADPH tetrasodium salt (Boehringer Mannheim), rabbit antiserum to cyclic GMP (Dr J. Garthwaite, University of Liverpool), collagenase type I (Worthington), cell culture materials (Flow and Gibco) and L-NMMA acetate (Wellcome) were obtained as indicated. All other reagents were obtained from either Sigma or BDH.

Statistics

Results are expressed as the mean $\pm$ s.e.mean for n experiments. Student's unpaired t test or analysis of variance followed by Fisher's least significant difference test to compare individual means were used, as appropriate. $P < 0.05$ was considered statistically significant.

Results

Effects of endotoxin on NO synthase in the left ventricular wall and plasma NO_x^-

Cytosols obtained from the left ventricular wall from rats injected with pyrogen-free saline showed a small but significant level of Ca^{2+}-dependent NO synthase activity (1.30 ± 0.16 pmol NO mg^{-1} protein min^{-1}, $n = 6$) which was not changed in cytosols obtained from rats at different times after injection of endotoxin (Figure 1a).

There was no significant Ca^{2+}-independent NO synthase activity in cytosols of left ventricular wall from rats 6 h after injection of pyrogen-free saline (Figure 1a). Thirty minutes

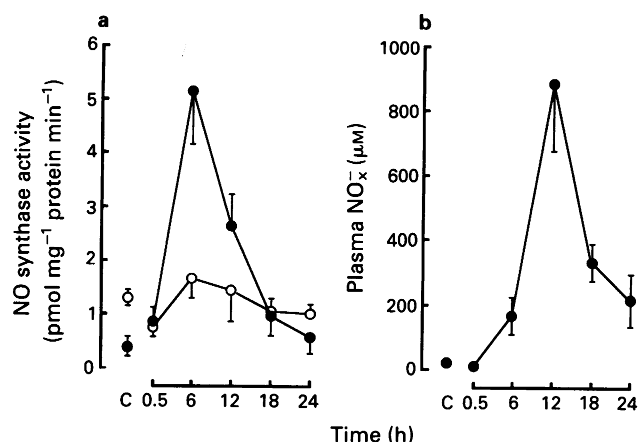

Figure 1 Time course of activation of left ventricular NO synthase and changes in plasma NO_x^- concentration after i.p. injection of endotoxin (LPS) or pyrogen-free saline in rats. (a) Ca^{2+}-dependent (○) and Ca^{2+}-independent (●) NO synthase activity in the left ventricular wall after treatment with LPS. C represents control values 6 h after injection of pyrogen-free saline. (b) Level of NO_x^- in plasma at time rats were killed after treatment with LPS. C represents control values 6 h after injection of pyrogen-free saline. Each point is the mean of heart or plasma samples from 4–6 animals; s.e.mean shown by vertical bars. If no error bars are shown the error lies within the height of the symbol.

after treatment with endotoxin, however, the level of Ca^{2+}-independent activity started to increase, reaching a maximum of 5.15 ± 1.00 pmol NO mg^{-1} protein min^{-1} ($n = 5$) at 6 h and declined thereafter towards the control level by 18 h (Figure 1a).

Rats injected with pyrogen-free saline had a plasma NO_x^- concentration of 22.6 ± 4.3 μM ($n = 7$) at the time when they were killed 6 h later (Figure 1b). Injection of endotoxin (4 mg kg^{-1}) caused a time-dependent increase in the concentration of NO_x^- in plasma which was significantly higher than the control value by 6 h, reached a peak of about 40 times the control value at 12 h and thereafter slowly declined towards control levels by 24 h (Figure 1b). The rise and fall in plasma NO_x^- coincided with the onset and disappearance of endotoxaemia-like symptoms in the rats, namely a huddled appearance, ruffled fur and lethargy, all of which were resolved by 24 h.

Six hours after treatment with endotoxin there was a 50% increase in the levels of cyclic GMP in the left ventricular wall compared with animals injected with saline (Figure 2).

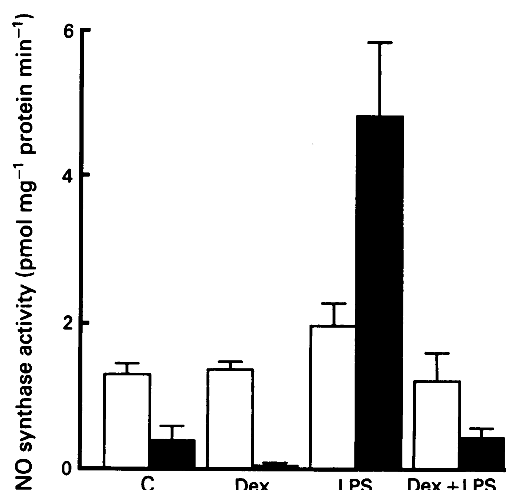

Figure 3 Effect of dexamethasone on the level of the Ca^{2+}-independent inducible (closed columns) and the Ca^{2+}-dependent constitutive (open columns) NO synthases in the left ventricular wall. The level of activity of the NO synthases 6 h after injection of pyrogen-free saline vehicle (C), dexamethasone (Dex), endotoxin (LPS), or LPS preceded 30 min by dexamethasone (Dex + LPS) is shown. The Ca^{2+}-dependent constitutive NO synthase activity was unaffected by the various treatments. LPS caused the expression of the Ca^{2+}-independent NO synthase activity which was abolished by dexamethasone. The results are the mean of values obtained from six animals per group; s.e.mean shown by vertical bars.

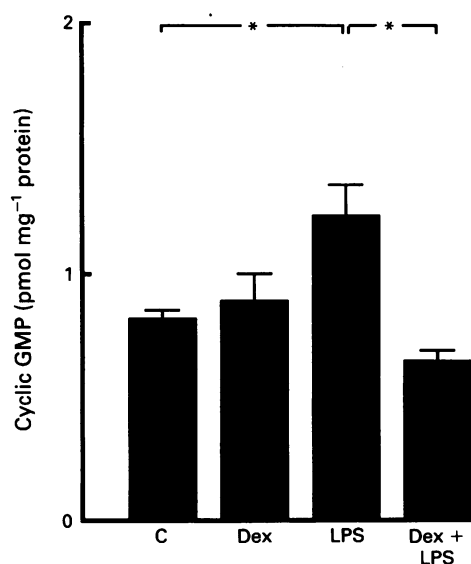

Figure 2 Effect of endotoxin (LPS) and dexamethasone on the level of cyclic GMP in the left ventricular wall 6 h after i.p. administration. Treatment with dexamethasone (Dex) had no effect on the basal level of cyclic GMP, whereas LPS raised the level of cyclic GMP significantly. Dexamethasone, given 30 min before LPS, abolished the increase in cyclic GMP by LPS (Dex + LPS). The columns represent the mean of samples taken from 4–6 animals; s.e.mean shown by vertical bars. * $P < 0.01$.

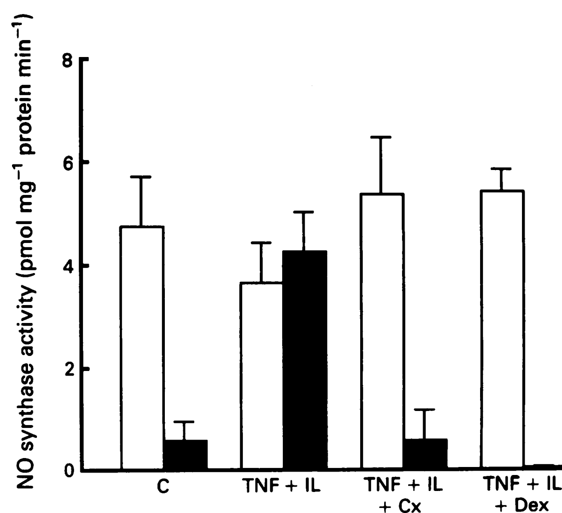

Figure 4 Constitutive and inducible NO synthase activity in isolated cardiac myocytes. Myocytes were kept for 24 h *in vitro* in control medium (C) alone or after various additions. Ca^{2+}-dependent constitutive NO synthase activity (open columns) seen in control myocytes is unaffected by treatment with tumour necrosis factor-α (TNF) and interleukin-1β (IL), alone or in combination with cycloheximide (Cx) or dexamethasone (Dex). In contrast, treatment with TNF and IL causes the expression of the Ca^{2+}-independent NO synthase (closed columns), which is abolished by Cx or Dex. The results are the mean of 3–5 experiments; vertical bars show s.e.mean.

Effect of dexamethasone

Treatment with dexamethasone alone did not significantly alter the plasma NO_x^- from the control level ($14.4 \pm 4.5\,\mu M$, $n = 6$, versus $22.6 \pm 4.3\,\mu M$, $n = 7$, respectively). Dexamethasone did, however, inhibit the increase in plasma concentration of NO_x^- induced by endotoxin from $361.6 \pm 39.1\,\mu M$ ($n = 6$) to $72.1 \pm 21.0\,\mu M$ ($n = 4$) at 6h. Furthermore, dexamethasone prevented the endotoxin-stimulated expression of the Ca^{2+}-independent NO synthase (Figure 3) and the increase in cyclic GMP in the myocardium (Figure 2) without affecting the activity of the Ca^{2+}-dependent enzyme (Figure 3).

NO synthase in freshly isolated cardiac myocytes

Cardiac myocytes incubated in control medium for 24h showed Ca^{2+}-dependent NO synthase activity of $4.73 \pm 0.99\,pmol\,mg^{-1}$ protein min^{-1} ($n = 4$) but no significant Ca^{2+}-independent NO synthase activity (Figure 4). Incubation with TNF-α and IL-1β induced the expression of Ca^{2+}-independent NO synthase activity in the myocytes which was prevented by cycloheximide ($10\,\mu M$), an inhibitor of protein synthesis, or by dexamethasone ($3\,\mu M$). The Ca^{2+}-dependent NO synthase activity was unaffected by treatment with the cytokines alone or in combination with cycloheximide or dexamethasone.

Discussion

Treatment of rats with endotoxin results, after a lag time, in the induction of a Ca^{2+}-independent NO synthase in the left ventricular myocardium. This enzyme, which is also expressed in isolated cardiac myocytes after treatment with TNF-α and IL-1β, is similar in properties and time course to that induced in many other cells and tissues in response to the same type of stimulus (Radomski *et al.*, 1990; Knowles *et al.*, 1990a; Rees *et al.*, 1990a; Busse & Mülsch, 1990; McCall *et al.*, 1991).

Depressed myocardial contractility in animals and man is known to occur in conditions associated with the release of cytokines, including endotoxin shock (Solis & Downing, 1966; Kadowitz & Yard, 1970; Gunteroth *et al.*, 1982; Velkov *et al.*, 1989; Suffredini *et al.*, 1989) or treatment with endotoxin *in vitro* (Macnicol *et al.*, 1973). Similarly, during antitumour therapy with cytokines (Deyton *et al.*, 1989; Nora *et al.*, 1989) or after exposure to cytokines *in vitro* (Hosenpud *et al.*, 1989; Hollenberg *et al.*, 1989; Sobotka *et al.*, 1990; Wiechmann *et al.*, 1991), a profound depression in cardiac function has been observed. Moreover, IL-1 and TNF have been shown to diminish the β-adrenoceptor-mediated increase in contractility in cultured cardiac myocytes (Gulick *et al.*, 1989). All these changes occur after a lag time of several hours suggesting that they involve *de novo* synthesis of protein.

Nitric oxide released from endocardial cells by the Ca^{2+}-dependent NO synthase (Schulz *et al.*, 1991) plays a role in the physiological modulation of myocardial contractility by increasing the level of cyclic GMP in cardiac muscle and thus exerting a negative inotropic effect (Smith *et al.*, 1991b; Shah *et al.*, 1991; Fort & Lewis, 1991). The mechanism for this is not clear; however, it may be due to the cyclic GMP-mediated inhibition of the entry of Ca^{2+} into the cell (Nawrath, 1977; Bkaily & Sperelakis, 1985; Hartzell & Fischmeister, 1986; Méry *et al.*, 1991) by activation of a cyclic GMP-dependent protein kinase (Méry *et al.*, 1991).

Induction by endotoxin or cytokines of a Ca^{2+}-independent enzyme in the myocardium leads to an excessive and prolonged release of NO which will result in diminished myocardial contractility. The negative inotropism in the heart during septic shock is accompanied by an increase in end-diastolic volume indicative of ventricular dilatation (see Parillo *et al.*, 1990), reflecting the change from a physiological (Ca^{2+}-dependent) release of NO by the endocardium to a pathological (Ca^{2+}-independent) NO release by the myocardium. This is comparable to the situation in the vascular wall, where the induction of a Ca^{2+}-independent release of NO in the endothelium and vascular smooth muscle leads to pathological vasodilatation (Rees *et al.*, 1990a; Smith *et al.*, 1991a; see Moncada *et al.*, 1991a for review). In this respect it is worth noting that we have recently observed the expression of a Ca^{2+}-independent enzyme in porcine endocardial cells *in vitro* (Schulz, Smith, Lewis and Moncada, unpublished observations).

Whether decreased contractility and ventricular dilatation are due to the stimulation of the soluble guanylate cyclase by NO (Moncada *et al.*, 1989) or to the direct cytotoxic actions of this molecule on iron-containing enzymes of the respiratory chain (Drapier & Hibbs, 1988), or both, is unknown. Our results show that the increased synthesis of NO in the myocardium is accompanied by an increase of 50% in the level of cyclic GMP. This moderate increase is comparable with a 90% increase in cyclic GMP levels observed in the rat isolated perfused heart 1 min after treatment with acetylcholine at a dose which produced a significant reduction in contractile response (George *et al.*, 1973). Further studies are required to determine whether, as in the vascular endothelium (Palmer *et al.*, 1992) and in EMT-6 adenocarcinoma cells (O'Connor & Moncada, 1991), prolonged induction of NO synthase in the myocardium leads to cytotoxicity.

Interestingly, we have observed a Ca^{2+}-dependent NO synthase activity in the myocardium and freshly isolated cardiac myocytes. Indeed, a continuous basal release of NO by this enzyme in the myocyte, acting on the guanylate cyclase, may regulate cardiac contractility in an autocrine fashion. This is different from vascular smooth muscle, which does not contain the constitutive NO synthase (Palmer *et al.*, 1987; Knowles *et al.*, 1990b; Rees *et al.*, 1990a; Busse & Mülsch, 1990) and in which the basal level of cyclic GMP is regulated by NO released from the endothelium (Rapoport & Murad, 1983; Martin *et al.*, 1986; Moncada *et al.*, 1991b). The physiological significance of this difference between cardiac and vascular muscle remains to be investigated.

Little is known, at present, about the consequences of inhibition of NO synthase on cardiac function. In the rat, administration of L-NMMA or other inhibitors of NO synthase leads to bradycardia, which is probably reflex in origin (Rees *et al.*, 1990b; Gardiner *et al.*, 1990). A negative inotropic effect has been observed following administration of L-nitroarginine methyl ester to the conscious rat; this may have been due to a direct action of this compound on the myocardium or to a reduction in coronary blood flow resulting from its potent vasoconstrictor actions (Gardiner *et al.*, 1990). More studies on the direct cardiac actions of inhibitors of NO synthase are required.

Induction of the myocardial Ca^{2+}-independent NO synthase by endotoxin caused a rise in plasma NO_x^- concentration, which followed the appearance of the enzyme in the ventricular wall. Inflammatory reactions *in vivo* are known to cause a marked increase in the excretion of nitrate (Wagner *et al.*, 1983; Stuehr & Marletta, 1985). Nitric oxide is rapidly oxidized in aqueous solution to nitrite (Palmer *et al.*, 1987), which is itself oxidized *in vivo* to nitrate (Witter & Balish, 1979). This is likely to occur by the stoichiometric reaction of nitrite with oxyhaemoglobin to form nitrate (Kosaka *et al.*, 1979). Urinary nitrate excretion was recently shown to be a sensitive indicator of the activity of NO synthase *in vivo* (Granger *et al.*, 1991). After endotoxin treatment, the NO synthase is induced in tissues other than the myocardium. Thus plasma NO_x^- levels reflect the production of NO in different organs as well as a balance between production and excretion of NO_x^-. The relative contribution of various tissues to the plasma level of NO_x^- and the fate of these metabolites remain to be studied.

Dexamethasone inhibited the induction of the Ca^{2+}-independent NO synthase in the myocardium and isolated

cardiac myocytes and the associated rise in myocardial cyclic GMP and plasma NO_x^- levels. The treatment of septic shock with glucocorticoids is controversial (Haynes & Murad, 1985). The benefits of these drugs in preventing the myocardial and haemodynamic consequences of septic shock are seen only if they are administered before endotoxin (Kadowitz & Yard, 1970). Interestingly, isolated papillary muscles taken from adrenalectomized rats are known to show a more rapid decline in contractile tension *ex vivo* than those from control animals and this can be prevented by treatment with dexamethasone *in vivo* or *in vitro* (Lefer, 1968). Whether the protection afforded by dexamethasone is due to its prevention of NO-stimulated increases in cyclic GMP levels or to inhibition of NO-mediated cytotoxicity (O'Connor & Moncada, 1991) is not yet known.

Our finding that endotoxin induces the myocardium to synthesize NO by a Ca^{2+}-independent enzyme is likely to have major consequences in our understanding of the cardiomyopathies associated with septic shock, inflammatory diseases of the heart such as endocarditis and myocarditis, idiopathic dilated cardiomyopathy, allograft rejection, burn trauma and anti-tumour therapy with cytokines (Deyton *et al.*, 1989). As endotoxin, exotoxin and infection with parasites have cardiac depressant effects similar to those seen in sepsis (Cunnion & Parillo, 1989) it may be that NO represents the final common pathway of disparate immunological challenges resulting in the pathophysiological alterations seen in these disorders. A better understanding of the two enzymes responsible for the synthesis of NO could lead to a selective inhibitor of the Ca^{2+}-independent NO synthase, which may prove useful in the treatment of these disorders.

We are indebted to Derek Smith for help in the preparation of the cardiac myocytes. We wish to thank Drs Richard Knowles and Mark Salter for helpful discussions and Annie Higgs and Gill Henderson for assistance in the preparation of the manuscript. R.S. is a fellow of the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research. E.N. is a fellow of the British Council.

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(Received July 25, 1991
Revised October 23, 1991
Accepted November 8, 1991)