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Depression of the inotropic action of isoprenaline by nitric oxide synthase induction in rat isolated hearts

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Abstract

The mechanisms involved in myocardial dysfunction during septic shock are not well understood. We have investigated the effects of endotoxin and the role of nitric oxide (NO) in the β-adrenoceptor responsiveness of rat isolated, ejecting hearts perfused at 60 mmHg of head pressure. In vivo pretreatment with endotoxin (4 mg/kg, i.p., 3 h before heart isolation) significantly attenuated the inotropic response (increase in left ventricular developed pressure, LVP) to isoprenaline (0.15 μg) after 30 min equilibration and after a further 90 min of perfusion. The peak rate of LVP development (dP/dt_{max}) in response to isoprenaline was reduced by endotoxin pretreatment, as was the increase of coronary flow. The depression of ventricular contraction was prevented by pretreatment with dexamethasone (1 mg/kg, i.p., 30 min before endotoxin), and was also restored by perfusion with N^G-nitro-L-arginine (L-NA, 10 μM) for 60 min, but not by N^G-nitro-D-arginine (D-NA, 10 μM). Mercaptoethylguanidine (MEG, 30 μM), a selective inhibitor of the inducible NO synthase (isoform 2), also reversed the depression of the isoprenaline response caused by endotoxin pretreatment. However, treatment with endotoxin, dexamethasone, L-NA, D-NA or MEG had minimal effects on the baseline parameters of LVP, dP/dt_{max} and coronary flow, which all tended to decline over the 2 h perfusion period. Western blot analysis using an antibody to NO synthase (isoform 2, but not to isoform 3) revealed the induction of a protein corresponding to NO synthase 2 in the endotoxin-treated hearts but not in control hearts or those treated with dexamethasone or MEG. In summary, these results indicate that endotoxin depresses myocardial contractile function and reduces inotropic responsiveness to β-adrenoceptor activation. The effect of endotoxin on the inotropic response is mediated, at least in part, by products of an endogenous NO synthase that is suppressed by dexamethasone and a specific inhibitor of NO synthase (isoform 2).

Keywords: Endotoxin; Nitric oxide (NO) synthase; Dexamethasone; Myocardial contractility; β-Adrenoceptor; Mercaptoethylguanidine

1. Introduction

Endotoxin shock is a major cause of death in patients suffering from septicemia. During septic shock the heart exhibits diminished contractility (Parker et al., 1990, 1984) that is characterized by decreased left ventricular ejection fraction and increased end-systolic and diastolic volume indexes. Isolated hearts from rats in septic shock and from endotoxin-treated rats and rabbits exhibit significant decreases in left ventricular pressure development (Smith and McDonough, 1988; Fish et al., 1985; Hung and Lew, 1993). Patients with septic shock suffer from hypotension and myocardial dysfunction refractory to high doses of intravenous β-adrenoceptor agonists. In endotoxicosis, decreased responsiveness to β-adrenergic stimulation has been observed in the heart (Bhagat et al., 1970; Romano and Jones, 1986; Romanosky et al., 1986; Shepherd et al., 1986). The decreased responsiveness to β-adrenergic stimulation in both patients with septic shock and in various animal models of sepsis suggests that endotoxin induces dysfunction of the β-adrenoceptor signal transduction pathway. Interleukin-1 and tumour necrosis factor, which can be released during endotoxin shock, have also been shown to diminish the β-adrenoceptor-mediated increase in contractility in rat cultured cardiac myocytes (Gulick et al., 1989). However, the mechanisms by which cytokines alter β-adrenoceptor responsiveness in these pathophysiological conditions are not fully elucidated.

Enhanced formation of NO following the induction of NO synthase (isoform 2) has been implicated in the pathogenesis of a number of conditions, including various forms of circulatory shock and inflammation (Kilbourn and Grif-

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fith, 1992; Szabó, 1995). In rats, a time-dependent induction of a Ca^{2+}-independent NO synthase activity occurs in cytosolic preparations of the myocardium after prolonged periods of endotoxemia and in isolated cardiac myocytes treated with cytokines (Schulz et al., 1992). In studies of rat cardiac myocyte contractility, the NO synthase inhibitor N^G-nitro-L-arginine (L-NA) was found to increase the inotropic effect of the β-adrenoceptor agonist isoprenaline on the myocytes but had no effect on basal contractility (Balligand et al., 1993b). Induction of NO synthase in rat ventricular myocytes attenuated the positive inotropic effects elicited by isoprenaline, suggesting that increased NO produced by myocytes may contribute to the contractile dysfunction characteristic of septic shock and some cardiomyopathies (Balligand et al., 1993a). The pathophysiological importance of over-production of NO by NO synthase (isoform 2) suggests that specific inhibitors of NO synthase have therapeutic potential, particularly those that do not interfere with the protective physiological roles of NO synthase in the endothelium.

The generation of NO can be inhibited by analogues of the substrate, L-arginine. However, the most commonly used inhibitors of NO synthase, such as N^G-methyl-L-arginine (L-NMA), L-NA and its methyl ester, inhibit the endothelial constitutive NO synthase (isoform 3) as well as isoform 2 (Gross et al., 1991; Lambert et al., 1991). Recently, compounds that are not amino acids, such as guanidines, *S*-alkylisothioureas and amidines have been reported to inhibit isoform 2 selectively. It has been shown that aminoguanidine, some *S*-alkyl-isothiothioureas, and some amidines express selectivity towards isoform 2 and have anti-inflammatory and anti-shock properties (Misko et al., 1993; Garvey et al., 1994; Southan et al., 1995). There are few published reports on in vivo experiments investigating the effects of these compounds in cardiovascular states associated with NO overproduction. Mercaptoethylguanidine (MEG) is a potent inhibitor of NO synthase 2 induced in homogenates of lung, but is a much weaker inhibitor of isoform 3 activity in homogenates of endothelial cells from bovine aorta (Southan et al., 1996).

In this study, we examined the effect of endotoxin on rat isolated, perfused hearts, and investigated whether any effect of endotoxin could be attributed to increased production of NO.

2. Materials and methods

2.1. Experimental preparation

Male Sprague-Dawley rats (250–350 g) were anesthetized with ether, and the hearts were rapidly excised and the left ventricle cannulated via the aorta. The hearts were then subjected to a modified Langendorff perfusion at a constant pressure of 60 mmHg with Krebs solution, containing (in mM) 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11 glucose, and were continuously gassed with 95% O_2/5% CO_2 to maintain pH 7.4. The perfusate was collected directly into a measuring cylinder for determining coronary flow rate (range 8–15 ml/min) for the variously treated hearts used. Drugs were slowly injected through a sidearm located upstream of the aortic cannula. A high-fidelity pressure transducer (Pneumatic-Transducer, DPT-6000, Peter von Berg Medizintechnik) was connected to the sidearm on the aortic cannula, and the aortic pressure signal was sampled at 2 kHz and fed via a bridge amplifier into a Maclab Chart v. 3.3.4 recording and analysis system (AD Instruments, Department of Physiology, Melbourne University).

Hemodynamic measurements performed during the study included the peak left ventricular developed pressure (LVP), the peak rate of rise of LVP (dP/dt_{max}), the peak rate of fall of LVP (dP/dt_{min}), heart rate and coronary flow rate.

2.2. Experimental protocol

After the equilibration period of 30 min, baseline LVP, dP/dt_{max}, dP/dt_{min}, heart rate and coronary flow were recorded. If these parameters were not stable, the heart was excluded from study. Krebs solution (without drug) or isoprenaline (0.15 μg) was injected as a bolus (0.5 ml) over 1 min, and hemodynamic changes observed over the next 5 min were recorded. Preliminary experiments established that this dose of isoprenaline was submaximal, and caused reproducible responses for up to 3 h of perfusion in control hearts. After washout of drug and re-equilibration for 15 min, the hearts were continuously perfused for a further 60 min with Krebs solution and a second dose of isoprenaline or Krebs solution was administered and the hemodynamic changes were recorded as described above.

Hearts were studied in six separate groups of rats as follows: (1) Control (no pre-treatment). (2) Endotoxin (lipopolysaccharide 4 mg/kg, pretreated i.p., 3 h prior to killing). (3) Dexamethasone (Dex; 1 mg/kg, pretreated i.p., 30 min before endotoxin). (4) and (5) Rats were pretreated with endotoxin and hearts received N^G-nitro-L-arginine (L-NA, 10 μM) and N^G-nitro-D-arginine (D-NA, 10 μM), respectively, perfused continuously beginning 30 min after the first dose of isoprenaline. (6) Rats were pretreated with endotoxin and hearts were perfused throughout in the presence of mercaptoethylguanidine (MEG, 30 μM).

2.3. Western blot analysis

At the end of the perfusion protocol, the heart ventricles were rapidly frozen with liquid nitrogen and then stored at −80°C for later processing. The ventricle tissue was homogenized in ice-cold homogenizing buffer containing 50 mM Tris-HCl, 1 mM EDTA and 0.1 mM EGTA. The homogenate was centrifuged at $100\,000 \times g$ for 30 min at

4°C and the cytosolic fraction was used for Western blot analysis. The total protein concentration was determined by Bradford assay. Equal total protein loads of 20 μg were loaded and separated on 8% polyacrylamide gels. Proteins were then transferred onto a nitrocellulose membrane (Bio-Rad Laboratories). Blots were blocked with 5% nonfat dry milk in TBS-T (20 mM Tris, 0.5 M NaCl and 0.05% Tween-20) for 1 h at room temperature. Incubation with the primary antibody (rabbit anti-macrophage NOS polyclonal IgG, Transduction Laboratories) against NO synthase (isoform 2) was at a dilution of 1:1000 at room temperature overnight and with the second antibody (horseradish peroxidase-conjugated sheep anti-rabbit immunoglobin, Amersham) at 1:1000 for 1 h. Specific proteins (130 kDa) were detected by enhanced chemiluminescence (Amersham). Wide-range color markers (Sigma) were used for molecular mass determinations. Two micrograms of mouse macrophage (J774.2) cell lysate stimulated with lipopolysaccharide (1 μg/ml) for 24 h was used as a positive control. A lysate of bovine coronary endothelial cells (20 μg) containing NO synthase (isoform 3) was used as a further control for the primary antibody to NO synthase (isoform 2).

2.4. Materials

Lipopolysaccharide (endotoxin) from *Escherichia coli* serotype 0111:B4, dexamethasone, N^G-nitro-L-arginine, and isoprenaline were all obtained from Sigma. N^G-Nitro-D-arginine was obtained from RBI, and mercaptoethylguanidine (MEG) was kindly provided by C.S.

2.5. Statistical analysis

For off-line analysis, the derived parameters were averaged over 5-s periods. Six of the 5-s averages obtained at 1 min intervals during the sampling period of 6 min were averaged to give a single value of each parameter for that point. Coronary flow rate was measured for the same period. The percent change from baseline was calculated. Data were reported as mean ± S.E.M. Student's paired *t*-test was used for within-group comparisons. Between-group comparisons were carried out by one-way analysis of variance (ANOVA) followed by Fisher's test. Significant differences were reported as $P < 0.05$.

3. Results

After an equilibration period of 30 min, baseline parameters immediately preceding each injection (at 30 min and 120 min perfusion) of Krebs solution or isoprenaline were monitored for a period of 10 min (Table 1). Baseline measurements of LVP, dP/dt_{max} and coronary flow in control and treated groups tended to show decreases over the 2 h perfusion time, with only some endotoxin-treated groups reaching significance (*t*-test, $P < 0.05$). Baseline heart rate remained unchanged over the 2 h perfusion time in all groups.

In this preparation, administration of Krebs solution as a bolus (0.5 ml) caused no significant changes in LVP, dP/dt_{max}, coronary flow and heart rate at either 30 min or 120 min.

3.1. The inotropic response to isoprenaline and the effect of endotoxin

In the control group of hearts, administration of isoprenaline (0.15 μg) induced a rapid (within 1 min) increase in LVP and dP/dt_{max}, which was reproducible after 30 min and 120 min of perfusion in control hearts (Figs. 1 and 2).

Table 1
Baseline parameters of ejecting hearts with different treatments

	Control	Endotoxin	Dex + endotoxin	Endotoxin + L-NA	Endotoxin + D-NA	Endotoxin + MEG
LVP, mmHg						
30 min	103 ± 3.0	93.8 ± 5.0	88.1 ± 5.0	95.3 ± 5.1	87.1 ± 5.9	92.7 ± 3.2
120 min	95.2 ± 5.8	85.5 ± 1.1	83.6 ± 3.1	85.9 ± 1.2	74.1 ± 1.8 [a]	79.3 ± 4.0 [a]
dP/dt_{max}, mmHg/s						
30 min	640 ± 52	689 ± 58	537 ± 46	695 ± 58	710 ± 41	486 ± 46
120 min	533 ± 100	440 ± 23 [a]	321 ± 34	456 ± 27 [a]	516 ± 103	290 ± 45 [a]
CF, ml/min						
30 min	11.7 ± 1.3	13.8 ± 1.2	13.6 ± 1.3	12.3 ± 1.1	10.7 ± 1.3	9.4 ± 0.3
120 min	13.7 ± 2.1	11.1 ± 0.9	9.3 ± 1.2	9.6 ± 1.1	8.9 ± 0.8	5.8 ± 1.1 [a]
HR, beats/min						
30 min	265 ± 15	274 ± 14	290 ± 27	301 ± 26	273 ± 17	226. ± 33
120 min	247 ± 17	296 ± 14	271 ± 20	303 ± 9	252 ± 14	187 ± 14

Values are mean ± S.E.M. $n = 4–5$ hearts per group. CF, coronary flow; HR, heart rate. [a] Significantly different from 30 min (Student's paired *t*-test, $P < 0.05$).

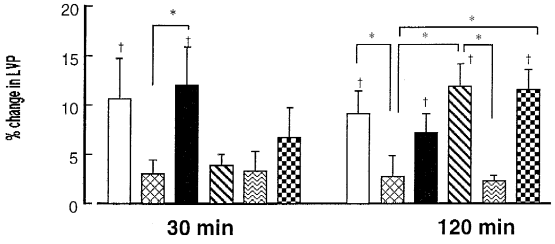

Fig. 1. Effect of different treatments on the increase of LVP produced by each injection of isoprenaline (0.15 μg) at 30 min and 120 min in perfused hearts. Values are mean ± S.E.M. Results are given as the percentage change from baseline value. First column: control, $n = 4$; 2nd column: endotoxin, $n = 4$; 3rd column: dexamethasone, $n = 4$; 4th column: L-NA, for 120 min only, $n = 4$; 5th column: D-NA, for 120 min only, $n = 5$; 6th column: MEG, $n = 5$. $^{+}$ Significantly different from baseline value (Student's paired t-test, $P < 0.05$). * Significant difference between groups at each time point (one-way ANOVA and Fisher's test, $P < 0.05$).

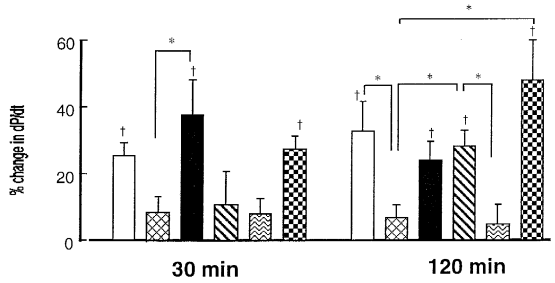

Fig. 2. Effect of different treatments on the increase of dP/dt_{max} produced by each injection of isoprenaline (0.15 μg) at 30 min and 120 min in perfused hearts. Values are mean ± S.E.M. Results are given as the percentage change from baseline value. First column: control, $n = 4$; 2nd column: endotoxin, $n = 4$; 3rd column: dexamethasone, $n = 4$; 4th column: L-NA, for 120 min only, $n = 4$; 5th column: D-NA, for 120 min only, $n = 5$; 6th column: MEG, $n = 5$. $^{+}$ Significantly different from baseline value (Student's paired t-test, $P < 0.05$). * Significant difference between groups at each time point (one-way ANOVA and Fisher's test, $P < 0.05$).

Pretreatment with endotoxin (4 mg/kg) 3 h prior to killing the rats significantly attenuated the inotropic response to isoprenaline as indicated by LVP and dP/dt_{max} measurements at 120 min, as compared to control hearts (ANOVA, $P < 0.05$). Similar effects were seen at 30 min, although these did not always reach significance (Figs. 1 and 2). Isoprenaline induced significant increases in coronary flow in control hearts, compared to baseline (t-test, $P < 0.05$), which was not seen in endotoxin-treated hearts at 30 min and 120 min (Table 2). Isoprenaline induced significant increases in heart rate in all groups, compared to baseline (t-test, $P < 0.05$) and the chronotropic action of isoprenaline was not significantly affected by endotoxin pretreatment (30 or 120 min; Table 2).

3.2. Effects of dexamethasone

Pretreatment with dexamethasone (1 mg/kg) 30 min before endotoxin administration prevented the attenuation of the response to isoprenaline in terms of LVP, dP/dt_{max} and coronary flow at 30 min and at 120 min, with the value of each parameter being similar to the level of the control group and significantly different from baseline (t-test, $P < 0.05$).

3.3. Effects of L-NA and D-NA

The reduction of the inotropic response to isoprenaline induced by endotoxin in isolated ejecting hearts was restored by perfusing for 60 min with Krebs solution containing L-NA, and the coronary flow response was significantly increased above the baseline, as in control and dexamethasone-treated hearts. In contrast, perfusion with D-NA did not alter the parameters of LVP, dP/dt_{max} or coronary flow, compared to endotoxin-treated hearts (Figs. 1 and 2). Neither treatment altered the chronotropic response to isoprenaline (Table 2, ANOVA, $P > 0.05$).

3.4. Effects of mercaptoethylguanidine

The inhibitory effect of endotoxin on the inotropic response to isoprenaline was partially reversed after 30 min perfusion with MEG and was completely reversed after 2 h perfusion with MEG (Figs. 1 and 2, ANOVA, $P < 0.05$). The baseline coronary flow declined significantly after 2 h perfusion with MEG, and the increase of flow induced by isoprenaline increased correspondingly,

Table 2
Effects of treatments on the response of coronary flow (CF) and heart rate (HR) to isoprenaline (0.15 μg)

	Control	Endotoxin	Dex + endotoxin	Endotoxin + L-NA	Endotoxin + D-NA	Endotoxin + MEG
Change in CF, ml/min						
30 min	0.6 ± 0.1 [a]	0.6 ± 0.6	0.5 ± 0.2 [a]	0.4 ± 0.2	0.3 ± 0.3	1.4 ± 0.5
120 min	1.8 ± 0.8 [a]	1.3 ± 0.8	1.0 ± 0.5 [a]	2.1 ± 0.4 [a]	−0.1 ± 0.2	3.5 ± 0.3 [a,b]
Change in HR, beats/min						
30 min	53 ± 9 [a]	23 ± 14 [a]	47 ± 27 [a]	42 ± 26 [a]	38 ± 17 [a]	61 ± 20 [a]
120 min	45 ± 14 [a]	64 ± 31 [a]	36 ± 11 [a]	25 ± 8 [a]	16 ± 3 [a]	78 ± 20 [a]

Values are mean ± S.E.M. $n = 4$–5 hearts per group. Results are given as changes from baseline value. [a] Significantly different from baseline value (Student's paired t-test, $P < 0.05$). [b] Significantly different from control, endotoxin alone, Dex + endotoxin and endotoxin + D-NA (one-way ANOVA followed by Fisher's test, $P < 0.05$).

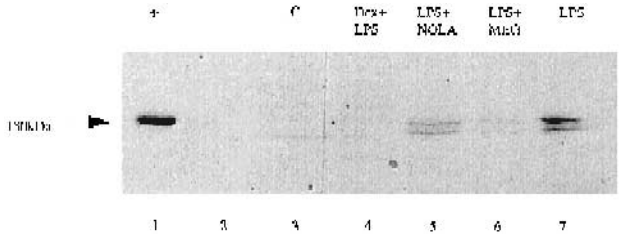

Fig. 3. Western blot analysis of NO synthase (isoform 2) protein expression induced by endotoxin (LPS) in rat heart. Hearts were treated with vehicle (C, lane 3), Dex + LPS (lane 4), NOLA + LPS (lane 5), MEG + LPS (lane 6) or LPS alone (lane 7). NO synthase 2 in macrophages was induced by LPS (see Section 2) as positive control (lane 1).

being significantly greater than in all the other groups (Table 2, ANOVA, $P < 0.05$).

3.5. Western blot analysis

Western blot analysis showed that untreated control hearts contained no detectable inducible NO synthase type 2 (Fig. 3; lane 3). In contrast, hearts treated with endotoxin contained a protein of approximatedly 130 kDa, which was recognised by a specific antibody to the inducible NO synthase 2 (Fig. 3). This induction of NO synthase 2 was prevented by dexamethasone and reversed or masked by MEG and reduced by N^G-nitro-L-alanine (NOLA). The NO synthase type 2 antibody did not reveal a protein in a standard preparation of NO synthase type 3 obtained from bovine coronary endothelial cells.

4. Discussion

The main finding of this study is that pretreatment of rats with lipopolysaccharide endotoxin reduces the inotropic effect of isoprenaline in the isolated heart, while baseline measures of ventricular contraction declined with continued perfusion after endotoxin. Heart rate responses were not altered. The glucocorticoid dexamethasone protected against the impairment of contractile function, and both non-selective and selective NO synthase inhibitors (L-NA and MEG, respectively) restored the endotoxin-depressed inotropic action of isoprenaline, although they were ineffective in reversing the steady decline in baseline contractile parameters in the same hearts.

Conditions associated with the release of cytokines, such as endotoxin shock, are known to depress myocardial contractility in animals and man (Solis and Downing, 1966; Kadowitz and Yard, 1970; Sufferedini et al., 1989). Antitumour therapy with cytokines likewise induces cardiac depression (Nora et al., 1989; Deyton et al., 1989). Treatment of cardiac tissues in vitro with endotoxin or cytokines has also been shown to depress myocardial contractility (Macnicol et al., 1973; Finkel et al., 1992), but in experiments with cat isolated papillary muscle (Kutner and Cohen, 1966) and guinea pig atrial muscle (Parker and Adams, 1979) endotoxin had no direct effect on contractile performance.

In more recent studies it has been shown that cytokines depress myocardial contractility indirectly by stimulating NO production. Sobotka et al. (1990) found that cultured human mononuclear cells, when stimulated with interleukin-2, produce a soluble factor that causes reversible, severe depression of cardiac function in rat isolated hearts. Schulz et al. (1995) recently reported that cardiac depression induced by interleukin-1β and tumour necrosis factor in rat isolated perfused hearts was prevented by continuous perfusion with L-NMA, and concluded this resulted from induction of a calcium-independent NO synthase. Moreover, using isolated cardiac myocytes from endotoxin-treated guinea pigs, Brady et al. (1992) demonstrated a significant reduction in the contractile responses elicited by electrical stimulation, which could be prevented by pretreatment of animals with dexamethasone, L-NMA or N^G-nitro-L-arginine methyl ester (L-NAME), again suggesting that induction of NO synthase was responsible. Finally, L-NMA blocked the negative inotropic effect of tumour necrosis factor, interleukin-1, and interleukin-6 in the isolated papillary muscle of the hamster (Finkel et al., 1992), although in this study the effects of the cytokines were too rapid to be explained by induced expression of a calcium-independent NO synthase. This raises the possibility that a constitutive, calcium-dependent NO synthase, which does exist in rat hearts (Schulz et al., 1995), might also contribute to depression of myocardial contraction.

In addition to depression of myocardial contractility, there is increasing evidence that endogenous NO suppresses the β-adrenoceptor signal transduction pathway in cardiac tissues. In adult rat cardiac myocytes the NO synthase inhibitor L-NA potentiated the inotropic response to isoprenaline, suggesting a role for a constitutive NO synthase in β-adrenoceptor responsiveness (Balligand et al., 1993b). The same group also showed that endotoxin-activated macrophages inhibited the positive inotropic action of isoprenaline in isolated ventricular myocytes and this inotropic response was restored by L-NMA, leading the authors to suggest that induction of NO synthase in myocytes was responsible for the myocardial dysfunction induced by endotoxin (Balligand et al., 1993a). In our study, the effects of endotoxin on inotropic action of isoprenaline were prevented by dexamethasone and reversed by L-NA, but not by D-NA perfusion, consistent with the suggestion that NO is responsible for this depressed function. We have now shown that the depression of the inotropic response to isoprenaline in rat hearts is also prevented by MEG, a specific inhibitor of the inducible NO synthase (isoform 2), at concentrations that do not affect endothelial constitutive NO synthase (isoform 3) in rat aorta (Southan et al., 1996). Moreover, both dexamethasone and MEG inhibited the expression of NO synthase 2 protein in the ventricular myocardium. Therefore,

it appears that in rat hearts endotoxin increases expression of NO synthase 2, and activity of NO synthase 2, and not that of a constitutive NO synthase, is responsible for endotoxin-induced suppression of the inotropic action of isoprenaline. Prevention by dexamethasone of the negative inotropic effects of endotoxin might also contribute to abrogation of the hypotension and hyporeactivity to pressor agents previously demonstrated in endotoxin shock in rats in vivo (Szabó et al., 1993).

An additional mechanism that might contribute to depression of cardiac contractility is the production of peroxynitrite, which may occur in the presence of superoxide anions (Beckman et al., 1990). Indeed, there is evidence that peroxynitrite is produced in the early stages of rat sepsis (Szabó et al., 1995), even before induction of NO synthase 2, and peroxynitrite might also depress cardiac contractility (Schulz and Wambolt, 1995). However, nitric oxide itself has been reported to reduce oxygen consumption and glycolysis in the canine heart (Shen et al., 1995), and there has been speculation that such uncoupling of myocyte metabolism might underlie the depression of cardiac contractility, especially in heart failure (Kelly et al., 1996). Further studies are clearly required to clarify these intriguing possibilities both in normal and in failing hearts.

The changes in coronary flow in our experiments are more difficult to interpret. Coronary resistance in this working heart is determined by the sum of metabolic vasodilatation (decreased with increased contractile activity), direct vasodilator actions of β-adrenoceptor stimulation (through cyclic AMP), vasodilator effects of NO released from the vascular endothelium and other constitutive NO synthases, vasodilatation resulting from excess NO generated by induced NO synthase 2, and interactions between these vasodilator mechanisms which may or may not be synergistic (Grace et al., 1988). Some general observations can be made. First, baseline coronary flow generally declines slightly with perfusion in all endotoxin-treated hearts, consistent with the decline in ventricular contractile activity. This decline is greater in the group treated with MEG, perhaps indicating that after endotoxin coronary flow is partly supported by excess NO derived from NO synthase 2. Second, in parallel with the inotropic response, isoprenaline causes an increase in coronary flow in all hearts except those treated with endotoxin or endotoxin plus D-NA, suggesting the flow response is largely metabolic in nature.

In contrast to the inotropic responsiveness, endotoxin and the other treatments had no consistent effect on the chronotropic response to isoprenaline. Ferguson and Vazquez (1984), using isolated perfused rabbit hearts, found differences in inotropic and chronotropic sensitivities to various β-adrenoceptor agonists, suggesting that myocardial β-adrenoceptors produce increases of heart rate and contractility through different receptor subtypes, or different transduction mechanisms. Similar results were obtained in the dog by Boucher et al. (1984). It is therefore possible that endotoxin and NO interact with different β-adrenoceptor transduction mechanisms subserving contractility and pacemaker activity.

In conclusion, our experiments indicate that endotoxin reduces myocardial contraction and inotropic responsiveness to β-adrenoceptor stimulation in the rat heart. This latter effect is mediated, at least in part, by products of an inducible NO synthase (isoform 2). Induction of NO synthase in the heart may have a role in the cardiac depression during shock and treatment with cytokines.

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