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Impaired vascular sensitivity to nitric oxide in the coronary microvasculature after endotoxaemia

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1 The effects of endotoxaemia on coronary vasodilator responses to bradykinin (BK), sodium nitroprusside (SNP) and nicardipine were investigated in the rat isolated heart perfused at constant flow *ex vivo*.

2 Dose-dependent reductions in coronary perfusion pressure reaching a maximum of 56 ± 3 and 57 ± 5 mmHg were observed for BK and SNP respectively. The BK response was biphasic, consisting of a rapid dilator response that was insensitive to N^Gnitro-L-arginine methyl ester (L-NAME, 0.1 mM) and a second slower component whose duration was attenuated by L-NAME.

3 Hearts obtained from rats treated with endotoxin (2.5 mg kg^{-1}, i.p.) for 2 or 6 h had increased basal coronary perfusion pressure and reduced vasodilator responses to BK or SNP. Dilator responses to nicardipine were not affected by endotoxin treatment. *In vitro* perfusion of hearts from endotoxin-treated rats with L-NAME (0.1 mM) restored SNP responses to control values.

4 Treatment with dexamethasone (1 mg kg^{-1}), 1 h before endotoxin did not alter the endotoxin-induced impairment of dilator responses to BK or SNP.

5 These results show that coronary microvascular responses are altered following endotoxin exposure. Endotoxin results in increased coronary microvascular tone despite induction of NO synthase and inhibits the dilator response to BK and SNP, vasodilators that act *via* the release of NO. Responses to SNP in endotoxin-treated hearts were restored to control values in the presence of L-NAME suggesting that enhanced endogenous NO synthesis might saturate guanylate cyclase resulting in reduced response to NO donors. The reduced response to vasodilators and increased coronary resistance might be important in determining the response of the coronary circulation to systemic inflammation and infection.

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Abbreviations: ADP, adenosine diphosphate; BK, bradykinin; L-NAME, N^Gnitro-L-arginine methyl ester; NO, nitric oxide; SNP, sodium nitroprusside; TNF, tumour necrosis factor

Introduction

The presence of endotoxin in the blood stimulates release of pro-inflammatory cytokines including tumour necrosis factor (TNF) and interleukin-1 and -6 (Dofferhoff *et al*., 1992). These cytokines impair vascular smooth muscle and myocyte contractility, at least in part, by inducing nitric oxide (NO) synthase which causes systemic vasodilatation and alters cardiac output (McKenna, 1990; Beasley *et al*., 1990; Finkel *et al*., 1992; Boillot *et al*., 1996). The end result is a state of hypotension and organ dysfunction known as septic shock (Parrillo, 1993).

Under normal conditions, endothelium-derived NO is an important regulator of coronary blood flow in the rat (Baydoun & Woodward, 1991), rabbit (Amezcua *et al*., 1988; 1989; Lamontagne *et al*., 1991), guinea-pig (Vials & Burnstock, 1992) and human (Lefroy *et al*., 1993; Kato *et al*., 1997). In the rat heart, acute exposure to endotoxin results in rapid NO production within 5 min, probably by releasing bradykinin, which stimulates endothelial NO synthase (Baydoun *et al*., 1993; Cannon *et al*., 1998). In contrast, exposure to endotoxin for longer times results in expression of inducible NO synthase (Liu *et al*., 1997) in the endothelium and smooth muscle and generation of large amounts of NO (Smith *et al*., 1991; Schulz *et al*., 1992).

It is generally accepted that these effects of endotoxin will lead to vascular relaxation and reduced responsiveness to vasoconstrictors. However it appears that, in rats, coronary blood flow, which is determined largely by microvascular resistance, is reduced in endotoxaemia (McDonough *et al*., 1985; Schulz *et al*., 1995). This might be due to increased vasoconstrictor release, or result from endothelial damage leading to impaired vasodilator production (Schulz *et al*., 1995). In rat aorta, rat mesenteric artery and cat coronary artery, exposure to the pro-inflammatory cytokine TNF-α results in impaired endothelium-dependent relaxation to acetylcholine and adenosine diphosphate (Lefer *et al*., 1991; Young *et al*., 1991; Greenberg *et al*., 1993; Wang *et al*., 1994; 1995; Fatehi-Hassanabad *et al*., 1996). Similarly in dogs, endotoxaemia impairs coronary smooth muscle function and endothelium-dependent relaxation (Parker *et al*., 1991; Parker & Adams, 1993), suggesting that a systemic inflammatory response paradoxically may decrease constitutive endothelial NO mediated dilatation in some situations.

The aim of the present study was to investigate the effects of endotoxin on blood flow. and vascular relaxation in the coronary microvascular circulation in the rat isolated perfused

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heart and to determine whether changes observed could be accounted for by endothelial dysfunction.

Methods

Materials

Wistar rats were obtained from Biological Research Facility, University College London. The composition of Krebs solution was (mM), NaCl 118; KCl, 4.5; $CaCl_2$, 1.4; $NaHCO_3$, 25; $MgSO_4$, 1.2; NaH_2PO_4, 1.4; glucose, 11. To increase coronary vascular tone hearts were perfused with Krebs solution containing 3.2 mM KCl. Lipopolysaccharide (*Escherichia coli*., serotype 055:B5) was obtained from Difco. Krebs solution was prepared with analytical grade salts (BDH, Dorset, U.K.). Dexamethasone sodium phosphate was obtained from Cox Laboratories, Ltd. N^Gnitro-L-arginine methyl ester (L-NAME), bradykinin, sodium nitroprusside, nicardipine, sodium nitrite and sodium nitrate were from Sigma Chemical Co. (Poole, U.K.) Bradykinin was prepared in 100% ethanol and stored at -20°C under nitrogen gas. All other drugs were dissolved in Krebs solution and prepared fresh each day.

Isolation and perfusion of the rat heart

Male Wistar rats (200–300 g) were stunned, exsanguinated and hearts rapidly removed and placed in ice-cold Krebs solution. Hearts were perfused using a modified Langendorff technique *via* the aorta at a constant flow rate of 10 ml min^{-1} with Krebs solution gassed with 95% O_2 and 5% CO_2. Coronary perfusion pressure was monitored continuously as an index of coronary microvascular tone with a pressure transducer attached to a side arm of the aortic cannula. Hearts were perfused with Krebs solution for 10 min after which this was changed to Krebs solution containing 3.2 mM KCl. This small reduction in extracellular potassium concentration raises coronary perfusion pressure in the rat heart thus allowing vasodilator responses to be observed (Criddle *et al*., 1991).

Experimental protocols

Dose responses to endothelium-dependent and -independent vasodilators were assessed by bolus injection (10 μl) of bradykinin, sodium nitroprusside or nicardipine into the coronary circulation, through a side port in the aortic cannula. Rats were treated with saline (1 ml kg^{-1}, i.p) or endotoxin (2.5 mg kg^{-1}, i.p.) and 2, 6 or 24 h later the animals were sacrificed and the hearts removed as described above. In some experiments rats received dexamethasone (1 mg kg^{-1}, i.p.) 1 h prior to administration of endotoxin. In each heart a single dose response curve first to bradykinin and then to sodium nitroprusside or nicardipine was constructed. Vasodilators were administered every 10 min or until the perfusion pressure had returned to pre dose values.

Determination of plasma nitrogen oxide levels

Total plasma nitrogen oxide (nitrite + nitrate) levels in rat plasma were determined by capillary electrophoresis as described by Leone *et al*. (1995). One-millilitre blood samples were obtained from control rats and those treated with endotoxin (2.5 mg kg^{-1}) by cardiac puncture into a heparinized syringe just before isolation of the heart for *in vitro* perfusion. Samples were centrifuged ($10{,}000 \times g$, 10 min), plasma removed and stored frozen at -70°C until later analysis.

For analysis, samples were diluted 1 : 10 with MilliQ$^+$ (18.2 MΩ resistance) water in the insert of Ultrafree MC filter (Millipore, mol weight cut off 5 kDa), ultrafiltered at $5000 \times g$ and analysed using a 3D capillary-electrophoresis system (Hewlett Packard) using 75 cm fused silica capillaries of 75 μm internal diameter. The electrolyte consisted of 25 mM sodium sulphate containing 5% NICE-Pak OFM Anion BT (Waters proprietary osmotic flow modifier) in MilliQ+ water. Samples were injected by electromigration (−6 kV, 20 s) and analysed at a negative potential of 30 kV. Standard curves for nitrite and nitrate were prepared using standard solutions of sodium nitrite and sodium nitrate prepared in MiliQ$^+$ water. The limit of detection for nitrite and nitrate was 1 μM.

Data analysis

Each experiment was replicated and the number, *n*, of experiments performed indicated as appropriate in the text. In control experiments rats were treated with saline and 2, 6 or 24 h later hearts were removed and responses to bradykinin or sodium nitroprusside assessed. No statistically significant difference was observed between these control groups and therefore the results were pooled into a single control group. Changes in coronary perfusion pressure were calculated from original trace recordings and expressed as mmHg. Responses to vasodilators were recorded as both the change in coronary perfusion pressure (mmHg) and the time taken for the perfusion pressure to return to 50% of its predose value ($t_{1/2}$, s). Differences between groups were analysed by one way analysis of variance followed by a Student-Newman-Keuls test with $P<0.05$ considered significant. Statistical analysis was performed using GraphPad Instat, San Diego. Data were plotted and curves fitted to dose-responses using Fig.P, Biosoft, Elsevier U.K.

Results

Effects of endotoxin on coronary perfusion pressure

In hearts obtained from control animals there was a small increase in coronary microvascular perfusion pressure over the first 10 min of perfusion after which it remained stable (Table 1). Reduction of extracellular potassium concentration resulted in an approximate doubling of coronary perfusion pressure (Table 1). In hearts obtained from animals treated

Table 1 Perfusion pressure in control and endotoxin-treated hearts

Time (min)	*Control* (mmHg) (n = *13*)	*2 h* (mmHg) (n = *5*)	*6 h* (mmHg) (n = *5*)	*24 h* (mmHg) (n = *4*)
1	41 ± 2	45 ± 1	51 ± 3*	41 ± 3
10	53 ± 2	99 ± 7***	83 ± 9***	46 ± 3
20	108 ± 3	98 ± 7	98 ± 4	116 ± 5

Hearts were obtained from animals treated with saline (1 ml kg^{-1}) or endotoxin (2.5 mg kg^{-1}) for 2, 6 or 24 h. Coronary perfusion pressure was measured at 1 and 10 min after commencement of perfusion with Krebs solution containing 4.5 mM potassium. After 10 min perfusate was changed to Krebs solution containing 3.2 mM potassium and all dilator dose-response curves were constructed under these conditions. Results are the mean ± s.e.mean of data obtained in 4–13 experiments. *$P<0.05$, ***$P<0.001$ versus control values.

with endotoxin (2.5 mg kg^{-1}) and removed 2 and 6 h later, the initial perfusion pressures (1 min) were similar to control hearts however during the first 10 min of perfusion the pressure increased to levels considerably higher than those seen in control hearts (Table 1). Reduction of extracellular potassium concentration did not increase further the perfusion pressure in hearts obtained from endotoxin-treated animals. Hearts obtained from rats treated with endotoxin and removed 24 h later behaved similarly to control hearts (Table 1). By reducing potassium to 3.2 mM in all experiments the perfusion pressure was similar for all dilator studies (Table 1).

Role of nitric oxide in bradykinin-induced vasodilatation

Bradykinin (BK, $10^{-14}-10^{-9}$ mol) resulted in a dose-dependent reduction in coronary perfusion pressure, the maximum reduction in pressure being 55 ± 3 mmHg ($n=13$, Figures 1A and 2A). The duration of response to BK was dose-dependent (Table 2). The contribution of endogenous nitric oxide release to BK-induced vasodilatation was determined by inhibition of NO synthase. Perfusion of control hearts with N^Gnitro-L-arginine methyl-ester (L-NAME, 0.1 mM, 20 min) resulted in a significant increase

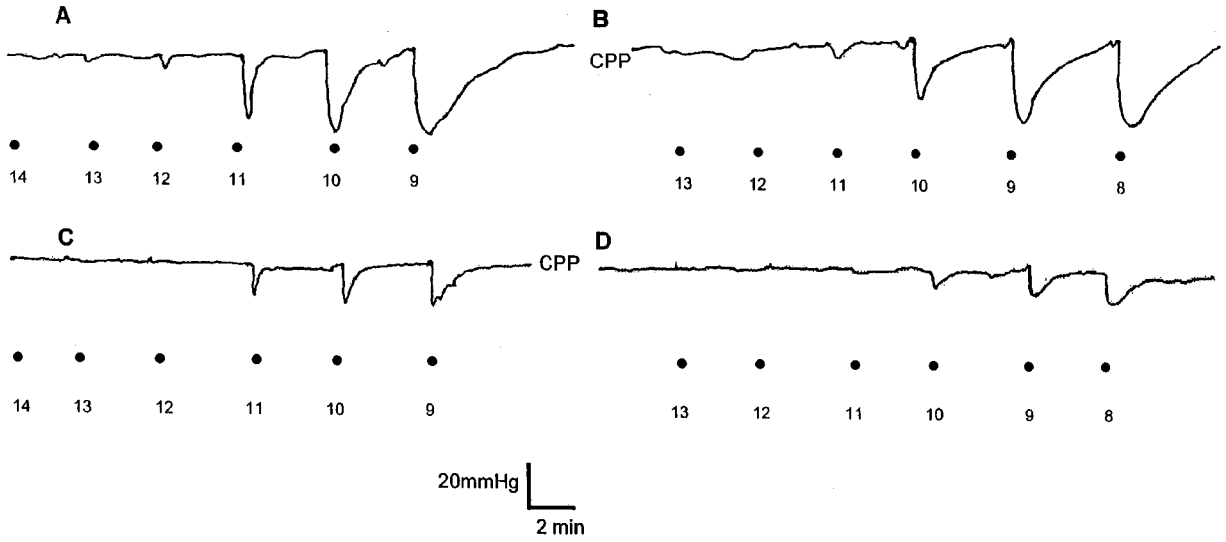

Figure 1 Coronary vasodilatation in control and endotoxaemic hearts. Original traces showing coronary vasodilator responses to bradykinin (BK) or sodium nitroprusside (SNP) in hearts from control rats (A and B, $n=13$) or rats treated with endotoxin (2.5 mg kg^{-1}, i.p, 6 h, $n=5$, C and D). Results are shown as the change in coronary perfusion pressure (CPP, mmHg) with respect to time (min).

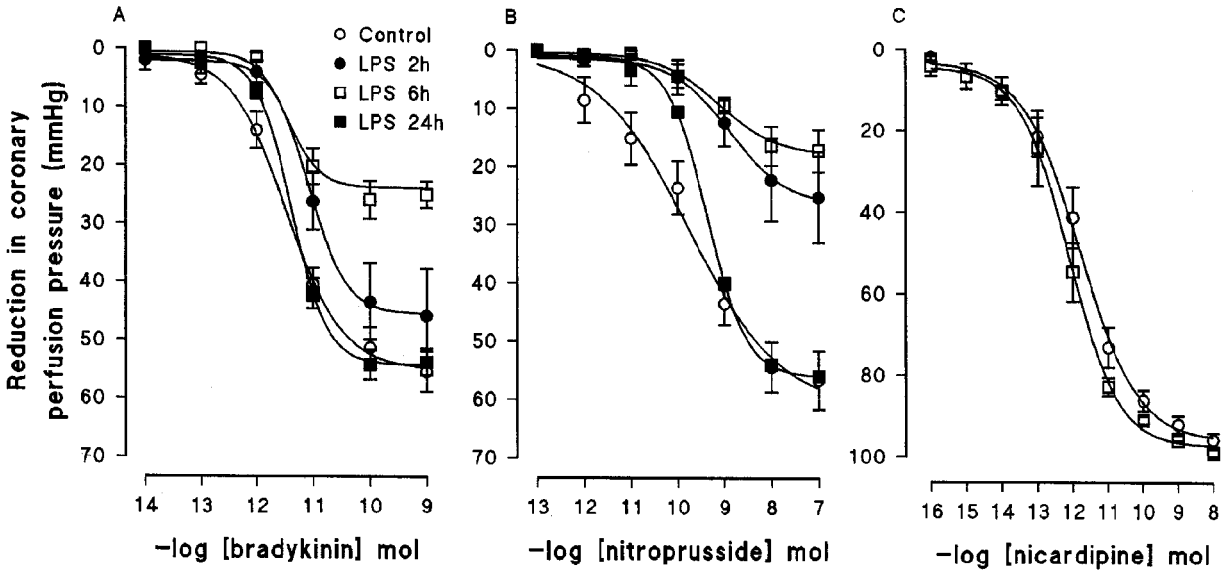

Figure 2 Effect of endotoxin on coronary vasodilator responses. Dose response curve to (A) bradykinin ($10^{-14}-10^{-9}$ mol, $n=13$), (B) sodium nitroprusside ($10^{-13}-10^{-7}$ mol, $n=5$) or (C) nicardipine ($10^{-16}-10^{-7}$ mol, $n=4$) were obtained in control hearts or those obtained from rats treated with endotoxin (2.5 mg kg^{-1}) and removed 2 h ($n=5$), 6 h ($n=5$) or 24 h later ($n=4$). Results are expressed as the maximum reduction in coronary perfusion pressure (mmHg) from baseline. Data are shown as the mean $\pm$ s.e.mean.

in coronary perfusion pressure from 105±3 to 138±7 mmHg ($P<0.01$; $n=3$). In the presence of L-NAME the immediate reduction in coronary perfusion pressure in response to BK (10^{-5} mol) was not different to that observed in control hearts different (54±3 versus 59±3 mmHg, $n=3$, $P>0.05$). However the time taken for the vasodilator response to return to 50% of its maximum was significantly shortened (99±9 s versus 25±4 s, $n=3$, $P<0.01$). In endotoxin treated animals L-NAME (0.1 mM) increased perfusion pressure by 34±4 mmHg from a baseline value of 114±5 mmHg ($n=5$).

Effects of endotoxaemia on responses to bradykinin and sodium nitroprusside

Treatment of rats with endotoxin (2.5 mg kg^{-1}) resulted in marked inhibition of the size and duration of vasodilator responses to BK (Figures 1A,C and 2A, Table 2). Significant inhibition of relaxation was observed at 2 and 6 h after endotoxin treatment. By 24 h, responses had returned to control values. Sodium nitroprusside (SNP) caused a dose-dependent reduction in coronary perfusion pressure with a maximum of 56±5 mmHg ($n=5$, Figures 1B,D and 2B). Treatment of rats with endotoxin resulted in an inhibition in the magnitude and duration of vasodilator response to SNP (Figure 1B and Table 2). The magnitude of response to SNP in hearts obtained from animals treated with endotoxin for 6 h were restored to control values during perfusion with Krebs solution containing L-NAME (0.1 mM; Figure 3). Similarly duration of response to SNP during L-NAME perfusion was not significantly different from control values (data not shown).

Effects of endotoxin on coronary responses to nicardipine

Nicardipine (10^{-14}–10^{-7} mol) resulted in a sustained reduction of coronary perfusion pressure which was similar in control hearts and those obtained from rats treated with endotoxin for 6 h ($n=4$; Figure 2C).

Effects of dexamethasone endothelium-dependent and -independent vasodilatation in hearts from endotoxaemic rats

Rats were treated with dexamethasone (1 mg kg^{-1}, i.p) 1 h before endotoxin (2.5 mg kg^{-1}, i.p). Six hours later hearts were removed and responses to BK and SNP were examined. Dexamethasone did not significantly alter the magnitude of the endotoxin-induced reduction in response to BK or SNP (Figure 4A,B). The duration of the response to SNP was increased in hearts obtained from animals pre-treated with dexamethasone compared to animals treated with endotoxin alone. This effect was only observed at higher doses of SNP (10^{6} mol: 49±6 versus 89±8 s and 10^{-5} mol: 114±13 s versus 162±14 s, $P<0.001$, $n=5$). There was no significant effect of dexamethasone on the duration of response to BK (data not shown).

Effects of endotoxin on nitrogen oxide levels in rat plasma

In control rats total plasma nitrate and nitrite was 9±1 μM ($n=4$). Treatment with endotoxin (2.5 mg kg^{-1}, i.p.) resulted in a time-dependent increase in plasma nitrate and nitrite levels (Figure 5). Injection of dexamethasone (1 mg kg^{-1}, i.p, 1 h) before endotoxin (2.5 mg kg^{-1} i.p. 6 h) significantly ($P<0.05$) reduced but did not completely suppress, levels of plasma nitrite and nitrate (Figure 5).

Discussion

Endotoxin treatment *in vivo* increased coronary microvascular resistance measured *in vitro* and reduced responsiveness to vasodilators whose actions are mediated through exogenous or endogenously generated NO. Endotoxaemia did not alter responses to the calcium channel antagonist nicardipine. The

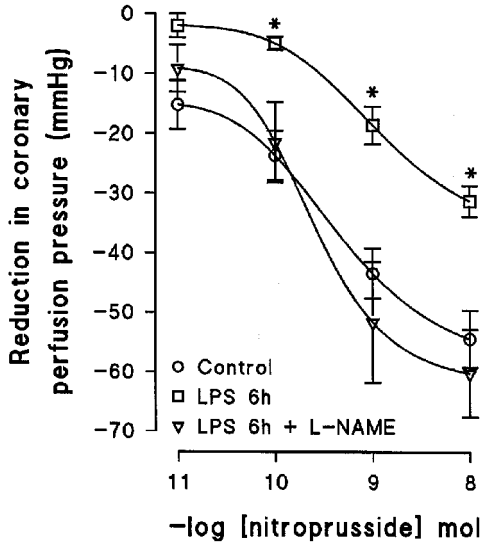

Figure 3 Effects of L-NAME on sodium nitroprusside induced coronary vasodilatation. Dose responses to sodium nitroprusside (10^{-11}–10^{-8} M) were constructed in hearts obtained from either control animals, rats treated with endotoxin (2.5 mg kg^{-1}, 6 h) or rats treated with endotoxin and perfused with Krebs solution containing L-NAME (0.1 mM). Results are the mean±s.e.mean of data obtained in four experiments. *$P<0.05$ versus endotoxin alone.

Table 2 Effect of endotoxin on duration of response to bradykinin or sodium nitroprusside

		Endotoxin treatment		
BK (mol)	*Control* (s)	*2 h* (s)	*6 h* (s)	*24 h* (s)
−14	3±2	5±4	0±0	0±0
−13	13±3	6±5	0±0*	5±2
−12	21±3	12±4	5±3*	18±2
−11	41±3	18±2***	19±4***	29±3
−10	98±10	54±5	44±10*	97±20
−9	179±20	72±9*	56±9**	153±29
SNP (mol)				
−13	0±0	0±0	0±0	0±0
−12	18±4	1±1***	1±1***	0±0***
−11	21±5	6±3*	5±3***	12±3
−10	49±6	28±12	16±5*	54±0
−9	142±38	40±11*	38±9*	54±0*
−8	199±42	82±40	49±6*	123±8
−7	247±35	155±60	114±13	195±14

Hearts isolated from animals treated with saline ($n=13$) or endotoxin (2.5 mg kg^{-1}) and removed 2 h ($n=5$), 6 h ($n=5$) or 24 h ($n=4$) later and perfused with Krebs solution. Responses to BK or SNP were assessed as previously described. The duration of response was measured as the time taken (s) for the vasodilator response to return to 50% of its maximum. Results are mean±s.e.mean with *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus control values.

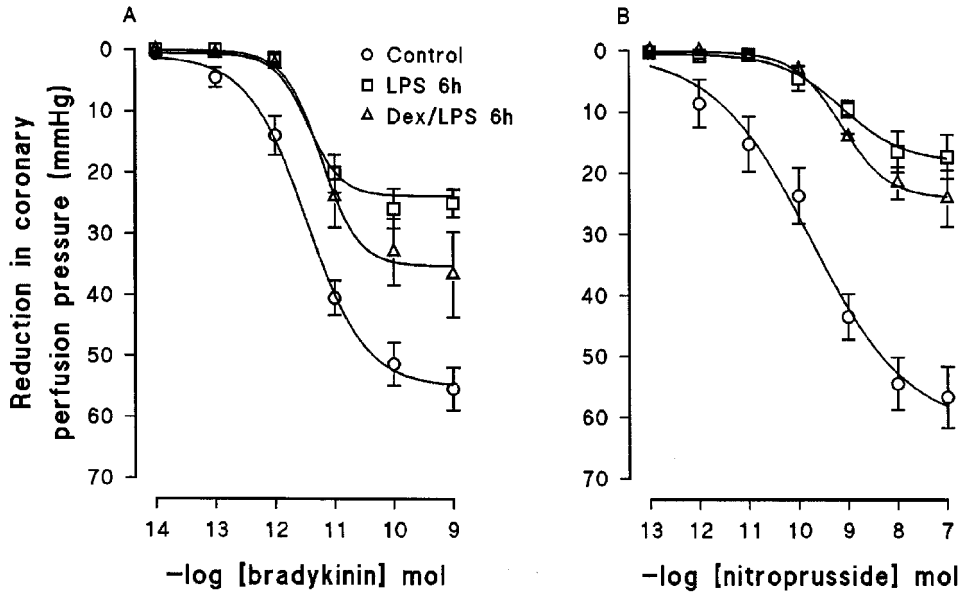

Figure 4 Effects of dexamethasone on responses to bradykinin and sodium nitroprusside by endotoxin. Hearts were obtained from rats treated with saline (1 ml kg^{-1}; control), or endotoxin (2.5 mg kg^{-1}, 6 h) or with dexamethasone (1 mg kg^{-1}, 1 h) followed by endotoxin (6 h). Dose response curves to bradykinin and sodium nitroprusside were constructed. Results are the mean ± s.e.mean of data obtained in four experiments.

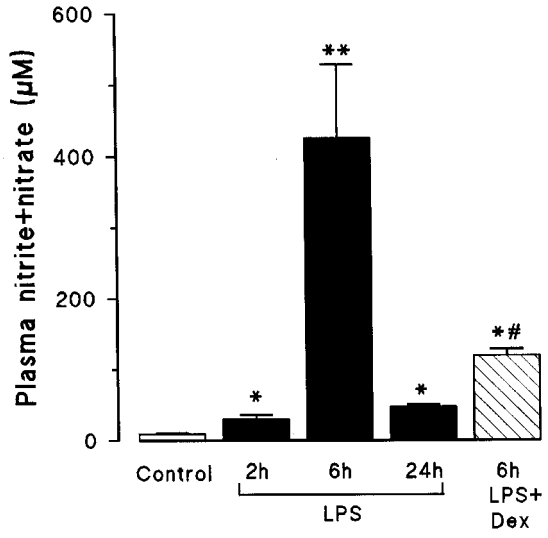

Figure 5 Effects of endotoxin on plasma nitrogen oxide levels. Rats were treated with endotoxin (2.5 mg kg^{-1}) for 2, 6 or 24 h or pre-treated with dexamethasone (1 mg kg^{-1}, 1 h) before administration of endotoxin. Subsequently, plasma samples were obtained by cardiac puncture and nitrogen oxides (nitrite and nitrate) were measured by capillary electrophoresis as described in the Methods section. Results are the mean ± s.e.mean of four experiments, $*P<0.05$, $**P<0.01$ versus control; $\#P<0.05$ versus 6 h LPS alone.

decreased sensitivity to exogenous NO induced by endotoxin was reversed by L-NAME but not by pre-treatment with dexamethasone.

Endotoxin-treatment increased coronary microvascular resistance and reduced the vasodilator response to bradykinin. This could reflect endothelial dysfunction and loss of basal vasodilator production or alternatively increased production of vasoconstrictors. In a previous study increased coronary microvascular resistance was observed after endotoxin treatment (Hohlfield *et al.*, 1995). The increased resistance was prevented by endothelin antibodies and reversed by endothelin antagonists (Hohlfield *et al.*, 1995; Klemm *et al.*, 1995), suggesting an important role for endothelin in the coronary vasoconstriction observed during sepsis. As we have shown following endotoxin-treatment, normal endothelial-dependent dilatation is functionally impaired, this might further enhance vasoconstriction tone and predispose to vasospasm.

The relaxation response to BK has at least two components, both of which are endothelium-dependent (Baydoun & Woodward, 1991). The first is a rapid dilatation that is probably mediated by lipid products and is sensitive to inhibitors of cytochrome P450 enzyme systems (Fulton *et al.*, 1996). The second phase of dilatation is slower and abolished by NO synthase inhibitors (our study, Baydoun & Woodward, 1991). We found that endotoxin treatment attenuated both components of the response to bradykinin. Our experiments with SNP showed that relaxations to exogenous NO were also attenuated by endotoxin. The impairment of this nitrovasodilator response followed a similar time course to the effect of endotoxin on BK relaxation. Possible mechanisms for reduced nitrovasodilator responses include impaired NO release from SNP; increase breakdown of released NO; or down-regulate/desensitization of guanylate cyclase secondary to enhanced endogenous NO production. After endotoxin exposure, induction of NO synthase occurs throughout the cardiovascular system including cardiac myocytes and coronary microvascular endothelium (Liu *et al.*, 1997). It is possible that elevated endogenous NO production resulted in saturation of soluble guanylate cyclase and an apparent impairment in the responses to endothelium-derived NO and exogenous NO donors. We found that the impaired relaxation to BK and SNP were maximal at a time when induction of NO synthase was greatest as assessed by plasma nitrite/nitrate levels although this reflects overall NO production averaged from all sites of production. To test the hypothesis that enhanced endogenous NO production impairs responses to exogenous NO in hearts from endotoxin-treated rats NO synthase was inhibited and responses to exogenous NO assessed. After treatment with L-NAME, responses to SNP were restored to values similar to those observed in hearts from control

animals. This observation is consistent with the idea that guanylate cyclase is near maximally active in hearts from endotoxin-treated rats due to high output NO production from inducible NO synthase. Application of exogenous NO or NO derived from constitutive NO synthase does not further activate soluble guanylate cyclase. The effect of L-NAME occurred over a short time course and is unlikely to be due to a change in the expression of guanylate cyclase. Further experiments with selective iNOS inhibitors might be useful to determine whether the endotoxin-induced impairment of bradykinin relaxation is restored following blockade of NO production from the inducible enzyme.

The time course of impaired responses to BK and SNP closely followed induction of NO as monitored by nitrogen oxides in the plasma of the animals. Maximum effects of endotoxin on coronary vascular responses were observed at 6 h and at this time NO production was markedly elevated. Despite the apparent inverse relationship between basal NO generation and responses to NO, pre-treatment of rats with dexamethasone suppressed markedly but not completely the generation of nitrite/nitrate in response to endotoxin but had only a small effect on impaired NO-mediated dilatation. This is similar to the observation that dexamethasone inhibits nitrite/nitrate production in rats without affecting LPS-induced hypotension (Hamilton & Warner, 1998). The reasons for this are not clear but it seems likely that the bulk of nitrite/nitrate measured during endotoxaemia comes from cells other than endothelial and smooth muscle cells (Cook *et al*., 1994). Our results may be consistent with substantial vascular NO generation continuing even when circulating nitrite/nitrate levels are only modestly elevated. Again the use of a selective iNOS inhibitor would help to test this hypothesis.

In addition to altering the NO-dependent component of the BK response endotoxin treatment attenuated the initial fast dilator response, which is NO-independent (Baydoun & Woodward, 1991). It is unlikely that loss of guanylate cyclase sensitivity to NO alone would produce this profile since L-NAME or the soluble guanylate cyclase inhibitor ODQ abbreviated the response to BK (our unpublished observations) but did not affect the initial component. The impairment of the early part of the BK response also mirrored the increase in systemic NO generation and it is possible that the large amounts of NO generated during endotoxaemia inhibited the transduction mechanism involved in this initial component of the BK response. This is conceivable since NO can inhibit certain P450 enzymes and NO synthase itself (Assreuy *et al*., 1993; Minamiyama *et al*., 1997). In guinea-pigs there is a selective loss of vasodilator responses to acetylcholine and ADP but not to A23187, substance P or NO donor (Parker & Adams, 1993). These results suggest that endotoxaemia may result in alteration of signal transduction mechanisms involving specific agonist-receptor coupling. Further studies will be required to investigate the mechanisms by which this fast initial component of the BK response is blocked by endotoxin.

The coronary response to endotoxin is species dependent. In dogs, endotoxaemia is associated with reduced cardiac output and impaired responses to endothelium-dependent vasodilators but not to NO donors are observed in large conduit vessels (Klabunde & Ritger, 1991; Parker *et al*., 1991). In the human heart coronary blood flow is increased during sepsis although there appears to be disordered coronary auto regulation and myocardial depression (Cunnion *et al*., 1986). Following inhibition of NO synthase there is a reduction in cardiac output, which may result from coronary vasoconstriction (Petros *et al*., 1994).

Our findings show that there is increased coronary resistance and impaired vasodilator responses to NO in hearts obtained from endotoxaemic rats. This may result in loss of local regulatory mechanism and an increased propensity towards coronary vasospasm, myocardial ischaemia and coronary dysfunction.

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