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SPECIAL REPORT

Coronary vasodilatation induced by endotoxin in the rabbit isolated perfused heart is nitric oxide-dependent and inhibited by dexamethasone

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The coronary vasoconstriction induced by the thromboxane mimetic U46619 (9, 11 dideoxy methanoepoxy 9α, 11α prostaglandin $F_{2\alpha}$, 3–30 nM) was significantly attenuated in hearts obtained from rabbits treated with endotoxin (lipopolysaccharide, LPS, 200 μg kg^{-1}, i.v.) 4 h before isolation of the heart. Under these conditions the vasoconstriction induced by two inhibitors of nitric oxide (NO) synthase, N^G-monomethyl-L-arginine (L-NMMA) and N-iminoethyl-L-ornithine (L-NIO) (1–100 μM for each) was significantly enhanced when compared to that induced in hearts from control animals. Both the decreased response to U46619 and the increased response to inhibitors of NO synthase were significantly attenuated by administration of dexamethasone (4 mg kg^{-1}, i.v.) 90 min before treatment with LPS. These data are consistent with the induction, by LPS, of an NO synthase, and the inhibition of this induction by dexamethasone. The enhanced NO synthesis contributes to the haemodynamic changes known to occur in endotoxin shock.

Keywords: Nitric oxide synthase; endotoxin; coronary circulation; dexamethasone; rabbit isolated heart

Introduction Endotoxin shock is characterized by hypotension and resistance to the actions of vasoconstrictors (Parratt, 1973; Suffredini *et al.*, 1989). Recently these changes have been attributed to the induction of an NO synthase which has been shown to occur in the endothelial and smooth muscle layer of rings of aortae exposed to endotoxin (lipopolysaccharide, LPS) *in vitro* (Rees *et al.*, 1990a) and *in vivo* (Knowles *et al.*, 1990). The NO synthase responsible for this pathological release of NO is distinct from the constitutive NO synthase in the vascular endothelium responsible for the physiological regulation of vascular tone and blood pressure (Moncada *et al.*, 1991). Both the constitutive and the inducible NO synthases are inhibited by the L-arginine analogues N^G-monomethyl-L-arginine (L-NMMA) and N-iminoethyl-L-ornithine (L-NIO) (Amezcua *et al.*, 1989; Rees *et al.*, 1990a,b). Furthermore, the glucocorticoid dexamethasone inhibits the induction of the inducible enzyme without affecting the activity of the constitutive enzyme (Rees *et al.*, 1990a; Knowles *et al.*, 1990).

In the present studies we have investigated the responses of the resistance vessels of hearts obtained from rabbits treated with LPS to the vasoconstrictor U46619 and to L-NMMA and L-NIO. We have also studied the effects on these responses of prior treatment of the animals with dexamethasone.

Methods Male New Zealand White rabbits (2.0–2.5 kg) were given LPS (*S. typhosa*, DIFCO, 200 μg kg^{-1}, i.v.). After 4 h the animals were anaesthetized and the heart isolated and perfused retrogradely at constant flow (25 ml min^{-1}) with Krebs buffer at 37°C gassed with 5% CO_2 in O_2 and containing indomethacin (5 μM), according to the method of Langendorff, as described previously (Amezcua *et al.*, 1988). Coronary perfusion pressure (CPP) was recorded as an index of vascular tone. The effect on CPP of 5 min infusions of U46619 (9, 11 dideoxy methanoepoxy 9α, 11α prostaglandin $F_{2\alpha}$), 3–30 nM, was recorded.

The response to L-NMMA and L-NIO (1–100 μM) was determined after adjusting the resting CPP to 35–45 mmHg with a continuous infusion of U46619. In some experiments animals were given dexamethasone (4 mg kg^{-1}, i.v.) 90 min prior to treatment with LPS. Data are expressed as the mean ± s.e.mean for *n* experiments and were analyzed by ANOVA with differences considered significant when $P < 0.05$.

Results The vasoconstrictor response to U46619 (Figure 1) was significantly reduced in hearts from animals treated with LPS when compared with hearts from untreated control animals. At 30 nM U46619, CPP increased by

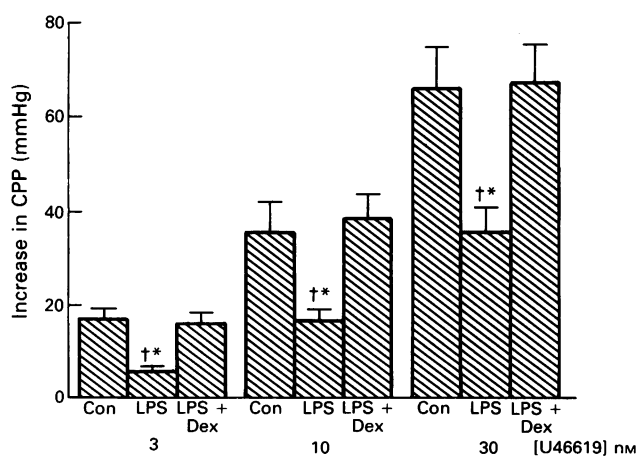

Figure 1 Effect of U46619 on coronary perfusion pressure (CPP, mmHg). U46619 (3–30 nM) caused a concentration-dependent rise in CPP in control hearts (Con) which was significantly attenuated by treatment of the animals with endotoxin lipopolysaccharide (LPS, 200 μg kg^{-1}). This effect was prevented by prior treatment with dexamethasone (4 mg kg^{-1}; LPS + Dex). Each column is the mean of 11–12 experiments; s.e.mean shown by vertical bars. †$P < 0.05$ against control and *$P < 0.05$ against LPS + Dex hearts. The basal CPP was 15.9 ± 1.9 mmHg for control hearts, 12.3 ± 0.8 mmHg for LPS-treated hearts and 13.9 ± 1.1 mmHg for dexamethasone-pretreated hearts (NS).

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35.8 ± 5.2 mmHg ($n = 12$) in LPS-treated hearts and by 66.2 ± 8.9 mmHg ($n = 11$) in control hearts. This effect of LPS was prevented by administration of dexamethasone before treatment with LPS (Figure 1). L-NIO (100 μM) caused a significant increase in CPP in control hearts (Figure 2a). This effect of L-NIO was significantly potentiated in hearts from LPS-treated animals, such that greater increases in CPP were observed at 1-100 μM L-NIO. Administration of dexamethasone before LPS treatment significantly attenuated this increased response to L-NIO (Figure 2a). Similar results were obtained with L-NMMA (1–100 μM, Figure 2b).

The increases in CPP induced by these concentrations of U46619 and L-NMMA were not significantly different in hearts from untreated animals or in hearts obtained from animals treated only with dexamethasone 5.5 h prior to isolation of the heart.

Discussion Treatment of rabbits with LPS leads to a reduction in the *ex vivo* response of the coronary circulation to the vasoconstrictor U46619 and to an enhanced response to L-NMMA and L-NIO, two inhibitors of NO synthase. These findings are similar to those observed in rat aortic rings exposed to LPS *in vitro* (Rees *et al.*, 1990a) and are consistent with the induction of an NO synthase in the wall of resistance vessels of the coronary circulation leading to an increase in the synthesis of NO and therefore to vasodilatation. The excessive NO thus formed functionally antagonizes the responses to U46619 and produces a greater response to L-NMMA and L-NIO, which constrict through inhibition of NO synthesis. The altered response to these compounds is prevented by prior administration of dexamethasone, which inhibits the induction of NO synthase in the rat aorta *in vitro* (Rees *et al.*, 1990a) and *in vivo* (Knowles *et al.*, 1990). Thus, our results provide further evidence to support the conclusion that the vasodilatation and the resistance to vasoconstrictors observed during endotoxin shock are attributable to the induction of an NO synthase in the vasculature and that the prevention of these haemodynamic changes by glucocorticoids is due to inhibition of the induction of this enzyme.

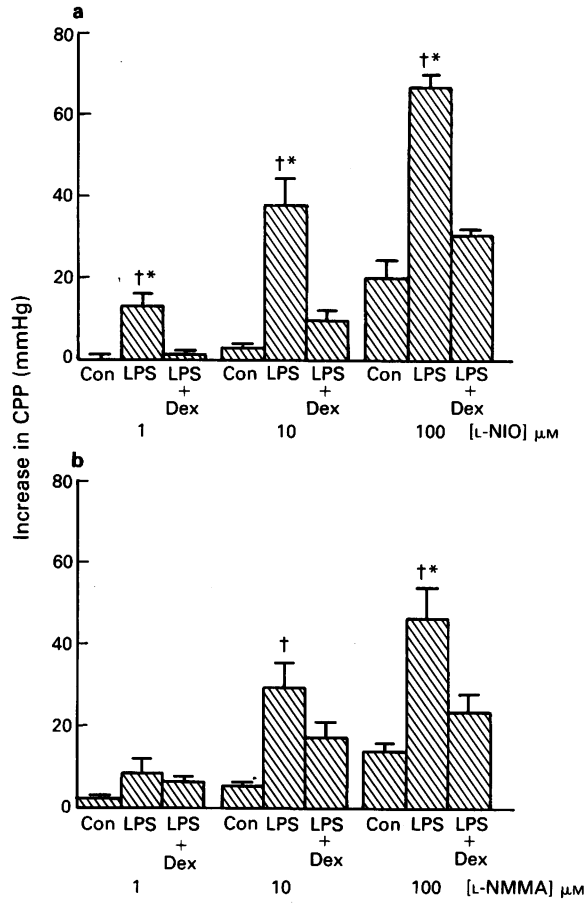

Figure 2 Effects of (a) N-iminoethyl-L-ornithine (L-NIO) and (b) N^G-monomethyl-L-arginine (L-NMMA) on coronary perfusion pressure (CPP mmHg). Both compounds (1–100 μM) caused a concentration-dependent rise in CPP (Con) which was significantly enhanced by treatment of the animals with endotoxin lipopolysaccharide (LPS, 200 μg kg^{-1}). This effect was attenuated to levels not significantly different from control values by prior treatment with dexamethasone (4 mg kg^{-1}; LPS + Dex). Each column is the mean of 4–5 experiments with L-NIO and 7–8 experiments with L-NMMA, s.e.mean shown by vertical bars. †$P < 0.05$ against control and *$P < 0.05$ against LPS + Dex hearts. Resting CPP in control hearts prior to infusion was 39.5 ± 1.3 mmHg ($n = 4$) for L-NIO and 38.3 ± 1.4 mmHg ($n = 7$) for L-NMMA and was not significantly different in the treatment groups.

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