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The effect of inhibitors of the L-arginine/nitric oxide pathway on endotoxin-induced loss of vascular responsiveness in anaesthetized rats

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1 The effects on blood pressure and on pressor responses to noradrenaline (NA), of N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME), inhibitors of the L-arginine/nitric oxide pathway, were investigated in anaesthetized rats receiving an infusion of bacterial endotoxin (*E. coli* lipopolysaccharide, LPS).

2 Infusion of LPS ($10\,mg\,kg^{-1}\,h^{-1}$) for 50 min had no effect on mean arterial blood pressure (MABP) but induced a reduction in responsiveness to noradrenaline ($100\,ng$–$1\,\mu g\,kg^{-1}$). L-NMMA ($30\,mg\,kg^{-1}$), but not D-NMMA, caused an increase in MABP of approximately 30 mmHg and restored responses to NA. This effect was reversed by L- but not D-arginine ($100\,mg\,kg^{-1}$).

3 In LPS-treated rats, blood pressure responses to NA were only marginally increased by the cyclo-oxygenase inhibitor, indomethacin ($5\,mg\,kg^{-1}$). L-NAME ($1\,mg\,kg^{-1}$) caused a similar increase in MABP and restored pressor responses to NA both in the presence and absence of indomethacin.

4 Co-infusion of vasopressin ($100\,ng\,kg^{-1}$, for 10 min) with LPS ($10\,mg\,kg^{-1}\,h^{-1}$) in order to reproduce the hypertensive effect of L-NMMA and L-NAME increased pressor responsiveness to 100 and $300\,ng\,kg^{-1}$ NA but not to $1\,\mu g\,kg^{-1}$ NA.

5 Infusion of sodium nitroprusside ($30\,\mu g\,kg^{-1}\,min^{-1}$) decreased responsiveness to NA even when the hypotension was corrected by co-infusion of vasopressin ($50\,ng\,kg^{-1}\,min^{-1}$).

6 These results demonstrate that the restoration of vascular responsiveness to NA in LPS-treated anaesthetized rats by inhibitors of the L-arginine/nitric oxide pathway is stereospecific and reversible. Furthermore, the experiments involving indomethacin suggest that although cyclo-oxygenase products of arachidonic acid may contribute to the development of LPS-induced hyporeactivity, the effect of L-NAME is unlikely to involve inhibition of the cyclo-oxygenase pathway. Comparison of NA responsiveness during vasopressin and L-NMMA/L-NAME-induced hypertension shows that increasing the blood pressure may modify LPS-induced hyporeactivity, but cannot account for the complete restoration of responses to NA by L-NMMA and L-NAME. These observations suggest that activation of nitric oxide formation from L-arginine makes a direct contribution to the production of vascular hyporeactivity by LPS *in vivo*.

Keywords: Endotoxin; vascular reactivity; L-arginine pathway; cyclo-oxygenase pathway; nitric oxide; L-NMMA; L-NAME; indomethacin

Introduction

Loss of vascular responsiveness to vasoconstrictor agents develops following administration, or release, of bacterial lipopolysaccharide (LPS) in animal models and may be an important factor contributing to eventual circulatory collapse. Cardiovascular hyporeactivity can be observed both *in vivo*, and *ex vivo* in vessels isolated from animals after LPS treatment. It affects the responses to a variety of constrictor substances including those acting independently of receptors (Parratt, 1989).

LPS is known to activate many cell types and administration to the whole animal sets in train the release of a variety of factors, which may be implicated as mediators of hyporeactivity. Among these we have recently proposed that nitric oxide (NO), derived from the amino acid L-arginine (Palmer *et al.*, 1988a) is a major mediator of the hyporeactivity seen both *in vivo* and *ex vivo* (Julou-Schaeffer *et al.*, 1990; Gray *et al.*, 1990a). This proposal was based largely upon the observation that responsiveness to constrictor agents in both systems was restored by N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of the L-arginine/NO pathway (Rees *et al.*, 1989a). Although the activity of L-NMMA and other related inhibitors has now been relatively well characterized *in vitro* (Moncada *et al.*, 1989; Rees *et al.*, 1990) and *in vivo* (Gardiner *et al.*, 1990a,b), their actions under pathological conditions like shock, have been less widely studied.

Underlying vascular tone is one of the factors which determines the response to vasoactive agents *in vivo* and this may be of particular importance after LPS administration when many vasodilator substances including prostacyclin, prostaglandins of the E series, plasma kinins and histamine, are liberated (Schlag & Redl, 1987). L-NMMA, in the dose required to restore responsiveness to noradrenaline also increased the blood pressure (Gray *et al.*, 1990b; Julou-Schaeffer *et al.*, 1990) probably through constriction of several vascular beds (Gardiner *et al.*, 1990a). At present the contribution of this increase in vascular tone compared to the inhibition of NO synthesis *per se* in the restoration of responsiveness to NA is unclear.

In a previous study L-NMMA prevented the development of LPS-induced hyporeactivity in anaesthetized rats, supporting our conclusion from *ex vivo* studies that activation of the L-arginine/nitric oxide pathway contributed to the induction of hyporesponsiveness by LPS (Julou-Schaeffer *et al.*, 1990). However, the possible contribution of indirect effects of L-NMMA to the restoration of responsiveness *in vivo*

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was not considered in this initial investigation. The aims of this present study were to characterize further the effect of L-NMMA in restoring responsiveness to noradrenaline *in vivo*, to examine its stereospecificity and reversibility and its potency compared to N^G-nitro-L-arginine methyl ester (L-NAME, a novel inhibitor of the L-arginine/nitric oxide pathway, Moore *et al.*, 1990), to investigate the role, if any, of a change in basal vascular tone and to consider the possibility of an interaction with the cyclo-oxygenase pathway of arachidonic acid metabolism. A preliminary account of some of these results has been given to a meeting of the British Pharmacological Society (Gray *et al.*, 1990b).

Methods

Anaesthesia was induced in male Wistar rats (12–15 weeks, 250–300 g) by intraperitoneal injection of sodium pentobarbitone (60 mg kg^{-1}) and maintained by intravenous injection as required. Arterial blood pressure was monitored with a pressure transducer (Gould Statham P23 ID) connected, via a cannula containing heparinised saline (100 iu ml^{-1}) to the right carotid artery. The output from the pressure transducer was displayed on a Beckman R511A pen recorder. Cannulae were also placed in the right and left femoral veins for administration of drugs, anaesthetic and LPS. The animals were allowed to breath spontaneously via a tracheal cannula. Body temperature was maintained at 37 ± 0.5°C with a heated underblanket controlled by a rectal thermistor probe.

Experimental protocols

After a stabilisation period of 20 min, pressor responses were obtained to noradrenaline (NA) 100 ng, 300 ng and 1 μg kg^{-1} (in 0.5 ml kg^{-1}). To assess the effect of LPS on NA-induced increases in blood pressure, responses were again obtained after 50 min of infusion of either LPS (10 mg^{-1} kg^{-1} h^{-1}) or its vehicle (saline, 0.4 ml h^{-1}). During continued infusion of LPS or saline the effects of various drug interventions were then investigated according to the protocols described below. The blood pressure was allowed to return to pre-NA levels between each drug.

Protocol 1 L-NMMA and L-arginine Pressor responses to NA were obtained 10 min after administration of L-NMMA (30 mg kg^{-1}), D-NMMA (30 mg kg^{-1}) or solvent (saline), again 5 min after D-arginine (100 mg kg^{-1}) or saline and then finally 5 min after L-arginine or saline.

Protocol 2 Indomethacin and L-NAME Pressor responses to NA were obtained 15 min after administration of indomethacin (5 mg kg^{-1}) or its solvent (4% bicarbonate) and then subsequently 10 min after L-NAME (1 mg kg^{-1}).

Protocol 3 Vasopressin Pressor responses to NA were obtained during a 10 min infusion of vasopressin (100 ng^{-1} kg^{-1} min^{-1} in LPS-treated and 50 ng^{-1} kg^{-1} min^{-1} in control rats) when the blood pressure had stabilised at approximately 30 mmHg above the pre-infusion level and then subsequently when blood pressure had returned to control levels on the cessation of infusion.

Protocol 4 Sodium nitroprusside In the absence of LPS or saline infusion, pressor responses to NA were obtained before infusion of sodium nitroprusside (SNP, 30 μg^{-1} kg^{-1} min^{-1}), during a 10 min infusion of SNP and then again on re-infusion of SNP with vasopressin (50 ng^{-1} kg^{-1} min^{-1}), which corrected its blood pressure lowering effect (see Results).

Drugs

Noradrenaline bitartrate, heparin sodium, N^G-nitro-L-arginine methyl ester hydrochloride, D-arginine hydrochloride and [Arg^8]-vasopressin acetate (all Sigma); L-arginine hydrochloride (Calbiochem); L- and D-N^G-monomethylarginine citrate (Salford Ultrafine chemicals); sodium nitroprusside (Merck) and lipopolysaccharide (*E. coli* 055:B5, Difco) were dissolved in 0.9% saline. Indomethacin (Sigma) was dissolved in a 4% solution of bicarbonate. Doses of drugs were calculated as g of salt per kg body weight.

Statistical analysis

Results are expressed throughout as arithmetic mean ± s.e.mean. Pressor responses to NA were compared by analysis of variance (ANOVA). Where ANOVA showed significant differences ($P < 0.05$) the results were further analysed with an *a posteriori* Student Newman-Keuls test.

Results

The effect of E. coli *lipopolysaccharide on mean arterial blood pressure and on pressor responses to noradrenaline*

Infusion of LPS (10 mg^{-1} kg^{-1} h^{-1}) over 50 min caused no significant change in mean arterial blood pressure (MABP) of anaesthetized rats (122 ± 3 mmHg before infusion and 117 ± 3 mmHg at 50 min, $n = 26$). Dose-dependent pressor responses to NA in these rats were, however, significantly reduced (Figure 1a and b). In control rats neither the MABP

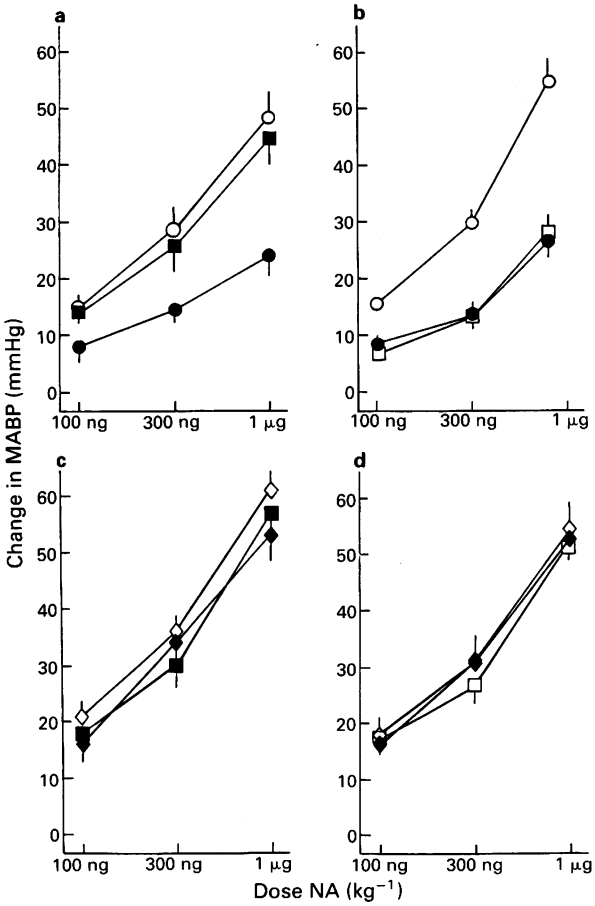

Figure 1 Effects of N^G-monomethyl-L-arginine (L-NMMA) and D-NMMA on increases in mean arterial blood pressure (MABP) elicited by noradrenaline (NA, 100 ng–1 μg kg^{-1}) during continuous infusion of *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1} h^{-1}, a and b) or saline (c and d) in anaesthetized rats. NA-induced pressor responses are shown before LPS (○) or saline (◇) infusion, after 50 min of LPS (●) or saline (◆) infusion and 10 min after administration of L-NMMA (■, 30 mg kg^{-1}, a and c) or D-NMMA (□, 30 mg kg^{-1}, b and d). Values are the mean of 6–7 experiments; vertical lines show s.e.mean.

(100 ± 5 mmHg before infusion and 103 ± 5 mmHg at 50 min, $n = 15$) nor pressor responsiveness to NA (Figure 1c and d) were altered by infusion of saline.

Effects of L-NMMA, D-NMMA, L- and D-arginine on mean arterial blood pressure and pressor responses to noradrenaline

Administration of L-NMMA (30 mg kg^{-1}) to LPS-treated rats resulted in a significant ($P < 0.01$) increase in MABP of 33 ± 5 mmHg ($n = 8$) and in the restoration of pressor responses to NA (Figure 1a). An equivalent dose of D-NMMA (Figure 1b) or saline (1 ml kg^{-1}, results not shown) had no significant effect either on MABP or on NA-induced pressor responses.

In control rats, infusion of L-NMMA increased the MABP to a similar degree as in LPS-treated rats (34 ± 6 mmHg, $n = 6$) while D-NMMA and saline were without effect. NA-induced increases in MABP were not altered by any of these interventions in control rats (Figure 1c and d, saline not shown).

During the continuous infusion of LPS (Figure 2), neither the L-NMMA induced increase in MABP nor the restored pressor responses to NA could be reversed by D-arginine (100 mg kg^{-1}) but both were reduced by the subsequent administration of L-arginine (100 mg kg^{-1}, Figure 2a). Both effects of L-NMMA were sustained until the end of the experiment if saline was injected in place of D- and L-arginine (Figure 2b). In the absence of L-NMMA, administration of L-arginine (100 mg kg^{-1}) alone caused no change in MABP or in pressor responsiveness to NA (Figure 2c). In control animals the L-NMMA-induced increase in blood pressure was reversed by L-arginine (MABP reduced from 142 ± 9 mmHg to 107 ± 14 mmHg, $P < 0.05$) but was unaffected by D-arginine or solvent (results not shown).

Effects of L-NAME and indomethacin on mean arterial blood pressure and pressor responses to noradrenaline

Because there is evidence of a common pathway for stimulation of nitric oxide and prostacyclin release in endothelial cells (de Nucci *et al.*, 1988), and for a role for cyclo-oxygenase products in mediating vascular hyporesponsiveness following LPS administration (Gray *et al.*, 1990c), indomethacin (5 mg^{-1} kg^{-1}) was administered during LPS infusion. This resulted in a significant increase in MABP (from 110 ± 6 to 125 ± 4 mmHg, $P < 0.02$). In addition, responses to NA 300 ng and 1 μg kg^{-1}, but not to 100 ng kg^{-1} were slightly but significantly ($P < 0.05$) increased after indomethacin (Figure 3a). The solvent for indomethacin (4% bicarbonate) was without effect on either MABP (105 ± 2 mmHg pre vs 109 ± 3 mmHg post) or on reduced responses to NA (Figure 3b). Subsequent administration of L-NAME (1 mg kg^{-1}) caused a similar increase in MABP in indomethacin pretreated (17 ± 1 mmHg) and in solvent pretreated rats (20 ± 4 mmHg) and restored pressor responses to NA to pre-LPS infusion values in both groups (Figure 3b). In saline-infused rats, neither indomethacin nor its solvent affected MABP or pressor responses to NA (results not shown). In these control rats, L-NAME increased the MABP (22 ± 4 mmHg in indomethacin pretreated, $n = 5$, 24 ± 3 mmHg in control, $n = 5$) but, like L-NMMA (Figure 1), had no effect on NA-induced pressor responses (see sample trace in Figure 4). For example, in rats not receiving indomethacin pretreatment increases in MABP in response to NA 100 ng, 300 ng and 1 μg kg^{-1} were 16 ± 2, 36 ± 3 and 52 ± 6 mmHg before L-NAME and 16 ± 4, 36 ± 3 and 53 ± 3 mmHg after L-NAME respectively ($n = 5$).

Effect of vasopressin on pressor responses to noradrenaline

In LPS-treated and in control rats vasopressin (VP) was infused at rates which induced similar increases in MABP to those observed with L-NMMA and L-NAME (see above). As in the previous groups LPS induced a reduction in pressor responsiveness to NA (Figure 5a), but no significant change in the MABP. During continuous LPS infusion a dose of 100 ng^{-1} kg^{-1} min^{-1} of VP was required to induce a change in MABP of 28 ± 2 mmHg. In control rats a similar increase in MABP (29 ± 7 mmHg) was induced by infusion of 50 ng^{-1} kg^{-1} min^{-1} of VP. This indicates that pressor responsiveness to VP as well as to NA was reduced by LPS infusion. During the infusion of VP in LPS-treated rats, pressor responses to 100 ng and 300 ng kg^{-1} of NA were slightly increased whereas those to 1 μg kg^{-1} NA were unaffected

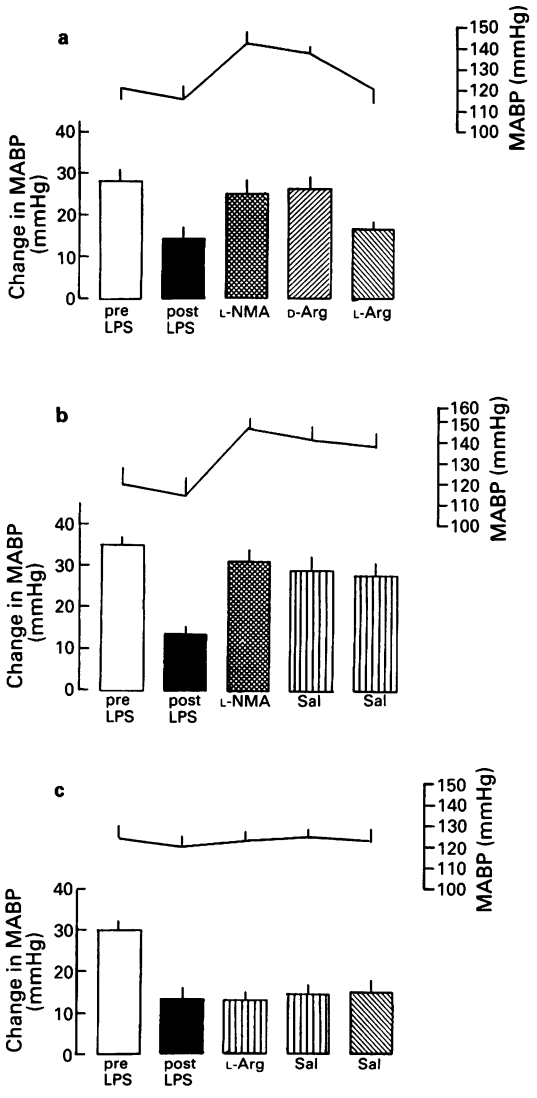

Figure 2 Stability of the effects of N^G-monomethyl-L-arginine (L-NMMA, 30 mg kg^{-1}) on MABP (line graphs) and pressor responses to noradrenaline (NA, 300 ng kg^{-1}, histograms) and their reversal by L- but not D-arginine (100 mg kg^{-1}) during continuous infusion of *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1} h^{-1}) in anaesthetized rats. The increase in MABP and restoration of responsiveness by L-NMMA (L-NMA) in (a) are both reversed by subsequent administration of L (L-Arg) but not D-arginine (D-Arg). (b) Shows them both maintained during saline (Sal) admininstration. In (c) both the MABP and LPS-induced depression of responsiveness are maintained during the time period of the experiment and MABP is not reduced, nor the hyporesponsiveness to NA enhanced, by provision of additional L-arginine (100 mg kg^{-1}). Values are the mean of 6–7 experiments; vertical lines show s.e.mean. * $P < 0.05$ compared to pre-infusion value.

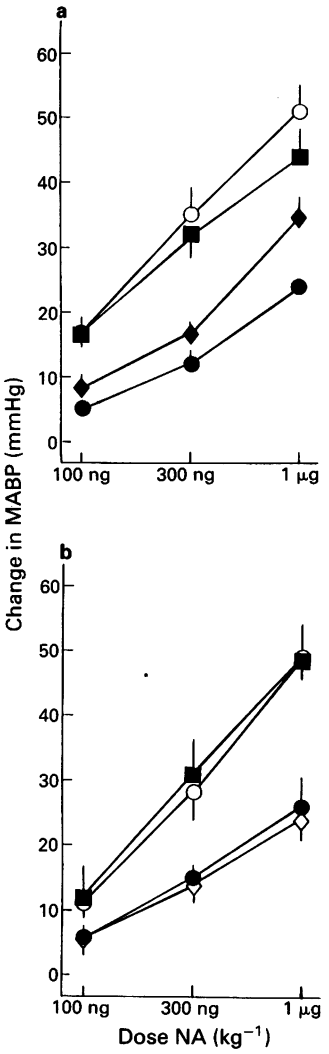

Figure 3 The effect of indomethacin (5 mg kg^{-1}) or its solvent (4% bicarbonate), and N^G-nitro-L-arginine methyl ester (L-NAME, 1 mg kg^{-1}) on pressor responses to noradrenaline (NA, 100 ng–1 μg kg^{-1}) in anaesthetized rats receiving a continuous infusion of *E. coli* lipopolysaccharide (LPS, 10 mg kg^{-1} h^{-1}, i.v.). NA-induced increases in mean arterial blood pressure (MABP) are shown before infusion (○), after 50 min of LPS infusion (●), 10 min after administration of indomethacin (◆, a) or its solvent (◇, b) and 5 min after the subsequent administration of L-NAME (■, a and b). Values are the mean of 6 experiments; vertical lines show s.e.mean.

(Figure 5a). VP was without effect on NA responses in control animals (Figure 5b).

Effect of sodium nitroprusside infusion on mean arterial blood pressure and pressor responses to noradrenaline

As in the experiments described above, NA produced a dose-dependent increase in the MABP of anaesthetized rats (Figure 6). Infusion of sodium nitroprusside (SNP, 30 μg^{-1} kg^{-1} min^{-1}) lowered MABP (from 120 ± 5 mmHg to 59 ± 1 mmHg, $P < 0.01$) and induced a significant reduction in these NA-induced pressor responses (Figure 6). On cessation of the SNP infusion both the MABP and NA responsiveness returned to normal (results not shown). To counteract the influence of the SNP-induced hypotension, it was co-infused with vasopressin (50 ng^{-1} kg^{-1} min^{-1}). Although the MABP was now similar to the pre-infusion level (118 ± 4 mmHg), pressor responsiveness to NA remained significantly depressed (Figure 6).

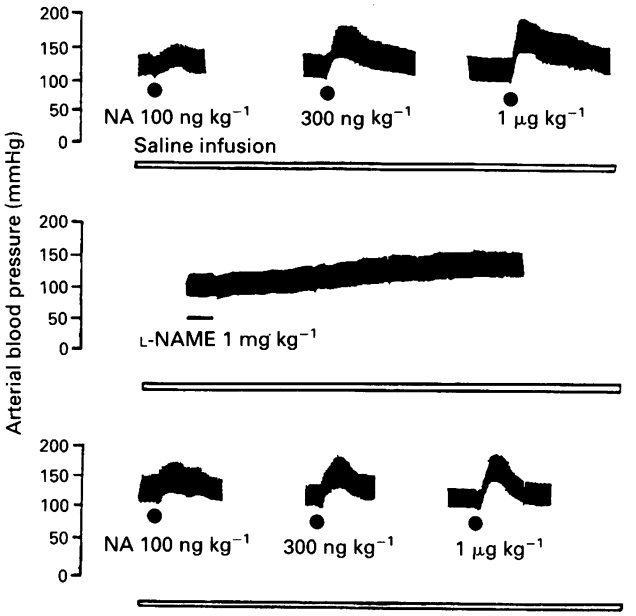

Figure 4 A representative trace showing the effect of N^G-nitro-L-arginine methyl ester (L-NAME, 1 mg kg^{-1}) on continuously recorded arterial blood pressure and pressor responses to noradrenaline (NA, 100 ng–1 μg kg^{-1}) in anaesthetized rats during infusion of saline (0.4 ml h^{-1}). Infusion of L-NAME over 1 min induced an increase in the basal blood pressure which was maintained during the administration of NA. Pressor responses to NA obtained 10 min after infusion of L-NAME were not altered from those obtained before L-NAME. This trace is representative of the data from 5 experiments, values are given in the text.

Discussion

In agreement with our previous findings (Gray *et al.*, 1990b; Julou-Schaeffer *et al.*, 1990) L-NMMA increased the mean arterial blood pressure and restored pressor responses to noradrenaline after infusion of LPS. The reversibility and stereospecificity of this effect is shown by the re-establishment of hyporesponsiveness by L- but not D-arginine and by the lack of effect of the D-enantiomer of NMMA, which does not inhibit synthesis of NO by endothelial cells (Palmer *et al.*, 1988b). Responsiveness was also restored by another analogue of L-arginine, L-NAME which has recently been shown to inhibit vascular nitric oxide synthesis (Rees *et al.*, 1990). It is of interest that this inhibitor was at least 30 times more potent than L-NMMA in both elevating blood pressure and in restoring reactivity to NA. This can probably be explained by the fact that L-NMMA but not L-nitroarginine undergoes substantial metabolism by the vascular endothelium to yield L-arginine, thus essentially becoming a substrate rather than an inhibitor (Hecker *et al.*, 1990). We have previously found that hyporesponsiveness to NA (Julou-Schaeffer *et al.*, 1990) and to calcium (Gray *et al.*, 1990a) in aortae removed from rats given LPS is enhanced in the presence of additional L-arginine. This implies that L-arginine is a rate limiting factor and that endogenous tissue supplies of the amino acid are depleted following LPS administration. However, in the present *in vivo* studies provision of L-arginine had no potentiating effect, suggesting that supplies of L-arginine are sufficient to allow full expression of the L-arginine/NO pathway. In this context, it is noteworthy that plasma levels of arginine are reported to be increased in the later stages of severe clinical septic shock (Cerra *et al.*, 1979).

Activation of the L-arginine/NO pathway was recently implicated in the induction of hypotension by endotoxin (Thiemermann & Vane, 1990) and by tumour necrosis factor, a cytokine released from macrophages in response to endotoxin (Kilbourn *et al.*, 1990). However, despite evidence for

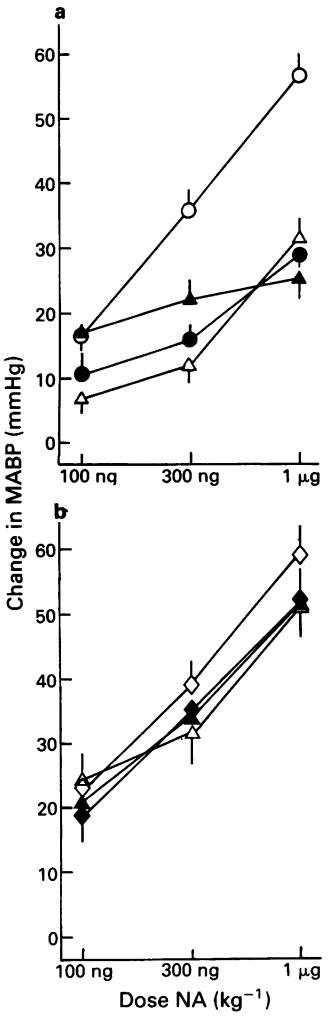

Figure 5 The effect of vasopressin, infused at doses to increase the mean arterial blood pressure (MABP) by approximately 30 mmHg, on pressor responses to noradrenaline (NA, 100 ng–1 $\mu g\,kg^{-1}$). (a) Increases in MABP elicited by NA before infusion of *E. coli* lipopolysaccharide (LPS) (○), after 50 min of LPS infusion (●, 10 $mg\,kg^{-1}\,h^{-1}$), during co-infusion of LPS with vasopressin (▲, 100 $ng^{-1}\,kg^{-1}\,min^{-1}$) and on cessation of vasopressin infusion (△). (b) Increases in MABP elicited by NA before infusion of saline (◇), after 50 min of saline infusion (◆), during co-infusion of saline with vasopressin (▲, 50 $ng^{-1}\,kg^{-1}\,min^{-1}$) and on cessation of vasopressin infusion (△). Values are the mean of 6–7 experiments; vertical lines show s.e.mean.

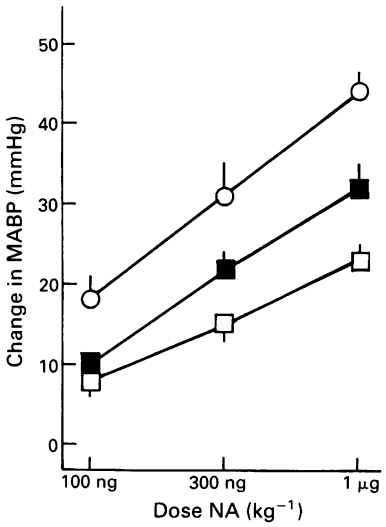

Figure 6 The effect of sodium nitroprusside (SNP, 30 $\mu g\,kg^{-1}\,min^{-1}$) alone, or co-infused with vasopressin (50 $ng\,kg^{-1}\,min^{-1}$, ■) on noradrenaline (NA, 100 ng–1 $\mu g\,kg^{-1}$)-induced increases in mean arterial blood pressure (MABP) in anaesthetized rats. Pressor responses are shown before SNP infusion (○), during infusion of SNP (□), and on co-infusion of SNP with a dose of vasopressin which corrected the SNP-induced hypotension. Values are the mean of 6 experiments; vertical lines show s.e.mean.

increased activation of this pathway by LPS infusion in the present study, there was no associated change in basal blood pressure. Hypotension can be produced by use of larger doses of LPS in anaesthetized rats, but a non-hypotensive dose was specifically chosen for the present study to simplify the comparison of NA-induced pressor responses between control and LPS-treated rats. It is likely that reflex compensatory mechanisms (McKechnie *et al.*, 1985) play a role in the prevention of hypotension. In pithed rats, which lack such reflex responses, hypotension is induced by much lower doses of LPS than that used here (Gray *et al.*, 1990c).

A paradoxical feature of the present results is that although L-NMMA and L-NAME caused equivalent increases in mean arterial blood pressure in control rats they had no effect on pressor responses to NA. Functional (Palmer *et al.*, 1988b) or mechanical (Furchgott & Zawadski, 1980) removal of the endothelium *in vitro* results in both an enhanced sensitivity and an increase in the maximal contractile responses to α-adrenoceptor agonists. This is believed to be due to inhibition of a basal release of relaxing factor/NO from the endothelium (Martin *et al.*, 1985). The pressor effect of L-NMMA *in vivo* has also been attributed to inhibition of this basal release (Rees *et al.*, 1989b; Aisaka *et al.*, 1989). Consequently, one might expect to see potentiation of *in vivo* responses to NA when there is functional impairment of the endothelium. The failure to demonstrate such an enhanced pressor response may be explained in several ways. Since responses to NA were measured in terms of changes in arterial blood pressure, it is possible that any potentiating effects of L-NMMA and L-NAME at the vascular level were masked by depression of cardiac output (through negative inotropic and chronotropic actions, Gardiner *et al.*, 1990b). However, the present dose of L-NAME was 10 times lower than that which reduced the cardiac output in the study of Gardiner *et al.* (1990b). Moreover, it remains true that L-NMMA and L-NAME enhanced NA-induced pressor responses in LPS-treated rats, in which they would also have caused a reduction of cardiac output. The results could also therefore be interpreted as showing that the basal release of NO from the endothelium is less important in determining responsiveness *in vivo* than *in vitro* or that L-NMMA and L-NAME are acting at a site removed from the endothelium to induce systemic hypertension. An examination of the effects of these inhibitors on NA-induced changes in regional vascular blood flow would be necessary to resolve the question of the role of basal NO release in determining vascular reactivity *in vivo*.

Whatever the mechanism, hypertension developed over a period of several min in response to both L-NMMA and L-NAME and was maintained for at least 30 min. Surprisingly the hypertension was of the same magnitude in both LPS-treated and control rats. These results contrast with those of a recent study in which L-NMMA-induced hypertension was significantly enhanced in anaesthetized dogs treated with tumour necrosis factor (TNF), a cytotoxic protein produced by macrophages on activation with bacterial endotoxin (Kilbourn *et al.*, 1990). A possible explanation for the discrepancy is that administration of TNF in the latter study was associated with a profound systemic hypotension whereas a non-hypotensive dose of LPS was chosen for the current experiments. The difference may reflect differences in degree of activation of the L-arginine/NO pathway. Alternatively, the doses of inhibitors used in the present study may have only submaximally inhibited NO production; certainly the dose of L-NMMA used in the present study (30 $mg\,kg^{-1}$), was found

to be submaximal in increasing the blood pressure in anaesthetized rabbits (Rees *et al.*, 1989b).

Because the hypertensive effects of L-NMMA and L-NAME are mediated largely by constriction in the renal, mesenteric and hindquarters vascular beds (Gardiner *et al.*, 1990a) we chose vasopressin, which causes a similar profile of vascular bed constriction (Gardiner *et al.*, 1989), to examine the role of the increase in basal blood pressure in restoring vascular responsiveness to NA during LPS infusion. Whilst this degree of hypertension had no effect on NA-induced pressor responses in control rats it slightly increased responses to the two lower doses of NA during LPS infusion. This result shows that although an increase in vascular tone may exert some influence in LPS-treated rats, it alone cannot account for the restoration of reactivity to NA by L-NMMA and L-NAME.

Prostaglandins have been extensively studied in models of endotoxaemia and septic shock and have been implicated in many of the pathophysiological sequelae (reviewed by Ball *et al.*, 1986). Indeed treatment with cylo-oxygenase enzyme inhibitors before either the induction of sepsis (Fink *et al.*, 1985) or infusion of LPS (Gray *et al.*, 1990c) prevents the development of hyporeactivity. In the present study, indomethacin given during LPS infusion in a dose which inhibits prostaglandin synthesis (Higgs & Flower, 1981) resulted in a marginal increase in the blood pressure and incomplete restoration of pressor responses to NA. This implies a limited contribution from vasodilator prostaglandins in determining vascular hyporesponsiveness, at least in this model. It does not exclude the possibility that indomethacin was indirectly blocking the release of NO. Prostaglandin E_2 (PGE_2) has recently been shown to increase LPS-stimulated NO synthesis by macrophage and Kupffer cell cytosol by increasing adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Mülsch *et al.*, 1990). The possibility that prostaglandins potentiate, or even initiate, the events leading to activation of the L-arginine/NO pathway by LPS accommodates the previous observations with inhibitors of cyclo-oxygenase (Fink *et al.*, 1985; Gray *et al.*, 1990c) and the current results with inhibitors of NO synthase. However, we found no evidence for the possibility that L-NAME increases blood pressure, or restores reactivity, through inhibition of prostaglandin synthesis in LPS-treated rats. Both these effects were the same after indomethacin or solvent pretreatment.

NO is believed to modulate vascular tone through stimulation of the guanylate cyclase enzyme in the cytosol of smooth muscle cells (Arnold *et al.*, 1977; Schultz *et al.*, 1977). The resulting accumulation of guanosine 3':5'-cyclic monophosphate (cyclic GMP), acting through several mechanisms (reviewed by Lincoln, 1989) reduces the availability of intracellular calcium to contractile proteins. The hyporesponsiveness to NA which occurred during infusion of sodium nitroprusside (which spontaneously releases NO and stimulates guanylate cyclase, Katsuki *et al.*, 1977) can be taken as further evidence for NO release by LPS. This has been confirmed in *ex vivo* experiments where we have found an increased concentration of cyclic GMP in aortae from LPS-treated, compared to control rats (Fleming *et al.*, 1990a). Moreover, contractile responses to NA (Julou-Schaeffer *et al.*, 1990) and to calcium in depolarizing solution (Gray *et al.*, 1990a) were restored by methylene blue, an inhibitor of soluble guanylate cyclase (Martin *et al.*, 1985). A role for cyclic GMP in production of hyporeactivity by LPS is a particularly attractive hypothesis given that it was proposed that the lesion produced by LPS involved post-receptor disruption of intracellular calcium regulation (Bigaud *et al.*, 1990).

While the present results suggest that NO production from L-arginine is increased after LPS administration, the cell type responsible remains to be determined. Evidence is now available for the presence of NO synthase in several of the many cell types activated by LPS, including macrophages (Hibbs *et al.*, 1987), neutrophils (Rimele *et al.*, 1989), endothelial cells (Mayer *et al.*, 1989; Mülsch *et al.*, 1989; Salvemini *et al.*, 1990a), mast cells (Salvemini *et al.*, 1990b), platelets (Radomski *et al.*, 1990), hepatocytes (Billiar *et al.*, 1990) and Kuppfer cells (Billiar *et al.*, 1989) all of which are potential sources *in vivo*. However, the demonstration of LPS-induced hyporeactivity in endothelium-denuded rat aortae (Fleming *et al.*, 1990b; Julou-Schaeffer *et al.*, 1990; Gray *et al.*, 1990a) would seem to exclude many of these cells and suggests a vascular source of NO. Indeed several authors have recently concluded that the L-arginine/NO pathway can occur in smooth muscle cells on the basis of experiments with endothelium-denuded aorta, either after L-arginine depletion (Wood *et al.*, 1990) or after removal from endotoxin-treated rats (Knowles *et al.*, 1990).

In summary, the present results show that neither the hypertensive effects of L-NMMA and L-NAME *per se* nor an interaction with cyclo-oxygenase products account entirely for the reversal of the LPS-induced vascular hyporeactivity by these inhibitors of the L-arginine/NO synthase pathway. These results and recent reports that NO contributes to TNF—(Kilbourn *et al.*, 1990) and endotoxin—(Thiemermann & Vane, 1990) induced hypotension therefore support our previous hypothesis that NO is a key mediator in endotoxaemia (Fleming *et al.*, 1990b; Julou-Schaeffer *et al.*, 1990).

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