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Vasoconstrictor responsiveness of tail arteries from endotoxaemic rats

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Abstract

Continuous infusion of lipopolysaccharide in conscious rats mimics some aspects of cardiovascular dysfunction in septic shock. In the present study, contractile responsiveness of tail arteries taken from rats infused with lipopolysaccharide was investigated. Contractile responses to α,β-methylene ATP and potassium chloride, but not to methoxamine, were greater after 24 h lipopolysaccharide infusion than in 2-h saline, 24-h saline and 2-h lipopolysaccharide groups. N^G-nitro-L-arginine methyl ester augmented contractions to α,β-methylene ATP and methoxamine in the 2-h saline, 24-h saline and 2-h lipopolysaccharide groups, but had no significant effect in the 24-h lipopolysaccharide group. Endothelium-independent vasorelaxant responses to sodium nitroprusside were greater in the 24-h lipopolysaccharide group compared to the other three groups. Relaxations to acetylcholine were not significantly different. In vitro incubation in medium containing lipopolysaccharide for 24 h had no significant effect on contractile responses of tail arteries compared to controls incubated in medium alone. These data indicate a possible impaired nitric oxide and/or endothelial function in tail arteries isolated from rats 24 h after lipopolysaccharide infusion. As hypercontractility was not evoked following in vitro incubation with lipopolysaccharide, the involvement of in vivo neurohumoral factors/mechanisms in the pathology of these changes is implicated.

Keywords: Endotoxaemia; Lipopolysaccharide; Tail artery; Vasocontraction

1. Introduction

Continuous infusion of lipopolysaccharide in conscious rats mimics some aspects of cardiovascular sequelea in septic shock, causing tachycardia and peripheral vasodilatation, and in this experimental model, at 2 and 24 h after the onset of continuous infusion of lipopolysaccharide, there is a reduced pressor and mesenteric vasoconstrictor responsiveness to methoxamine given intravenously (Waller et al., 1994; Tarpey et al., 1998). Moreover, impaired vasoconstriction to methoxamine in aortae and mesenteric arterial beds isolated from rats following 24 h lipopolysaccharide infusion has been reported (Farmer et al., 2001, 2002), indicating that the hypocontractility involves changes in these blood vessels that persist ex vivo. As impaired responsiveness to phenylephrine occurs in rat isolated aortae and mesenteric arteries incubated for 6 or more hours with lipopolysaccharide (Hall et al., 1996; O'Brien et al., 2001), it seems that, at least in these blood vessels, a local action of lipopolysaccharide is involved. We have previously shown that a vascular bed (mesenteric arterial bed) and conduit artery (aorta) taken from rats treated with lipopolysaccharide for 2 and 24 h showed different temporal changes in sensitivities to methoxamine (Farmer et al., 2001, 2002), and thus were interested to see what type of change in contractile responsiveness, if any, occurs in the tail artery in endotoxaemia.

The mechanisms underlying endotoxin-induced vascular hypocontractility are complex and not fully understood, but induction of nitric oxide synthase is believed to be involved (Parratt, 1998; Boyle et al., 2000; O'Brien et al., 2001). Accordingly, inhibition of nitric oxide synthase or soluble guanylyl cyclase (blocking the formation of cyclic cGMP and vasorelaxation) has been shown to reverse vascular hyporeactivity in vivo and in vitro (Julou-Schaeffer et al., 1990; Mitolo-Chieppa et al., 1996; Hall et al., 1996; O'Brien et al., 2001). Inducible nitric oxide synthase is not the only mechanism involved as hypotension and vasodilatation caused by lipopolysaccharide in conscious rats

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was resistant to aminoguanidine (a selective inhibitor of inducible nitric oxide), and these haemodynamic changes were not always associated with elevations in tissue activity of inducible nitric oxide synthase (Gardiner et al., 1995, 1996). Additional mechanisms, including induction of cyclo-oxygenase and enhanced activation of potassium channels are likely to be involved (Hall et al., 1996; Bishop-Bailey et al., 1997).

In the present study, responsiveness to methoxamine and other vasoactive agents, in Krebs' perfused tail arteries isolated 2 and 24 h following the start of lipopolysaccharide infusion in rats, was investigated. There is evidence for an enhanced [^{3}H]-noradrenaline release, in the presence of L-arginine, from sympathetic nerves in tail arteries removed from rats treated with lipopolysaccharide (Ohlmann et al., 2000), although the noradrenaline content is reported to be decreased (Wang et al., 2000). Relatively little is known, however, about the effect of endotoxaemia on vascular responsiveness of the tail artery. Since the mode of administration of a contractile agonist (bolus injection versus continuous infusion) may influence responsiveness in vitro (Farmer et al., 2001), both routes were investigated for methoxamine administration in the present study. In addition, as the nature of the vascular change can depend on the agonist used, we investigated the effects of two other vasoconstrictors (which act independently of G proteins), namely, α,β-methylene ATP, an agonist at ionotropic P2X receptors, and potassium chloride.

In preliminary studies, we observed that contractile responses to α,β-methylene ATP were augmented in tail arteries isolated from rats 24 h following onset of lipopolysaccharide infusion (Farmer et al., 2002). In order to investigate the possible involvement of nitric oxide in the changes we observed, we used N^G-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase. Endothelial function was further investigated using acetylcholine and sodium nitroprusside, endothelium-dependent and -independent vasodilators, respectively. Finally, the possible involvement of local actions of lipopolysaccharide in the hypercontractility observed to α,β-methylene ATP and potassium chloride was investigated by incubation of isolated tail arteries for 24 h in culture medium containing lipopolysaccharide (O'Brien et al., 2001), thereby eliminating an involvement of any neural or humoral factors generated elsewhere in the body.

2. Methods

2.1. Induction of endotoxaemia

Under anaesthesia (fentanyl and medetomidine; 300 μg kg^{-1} of each i.p.) male Sprague–Dawley rats (300–419 g)

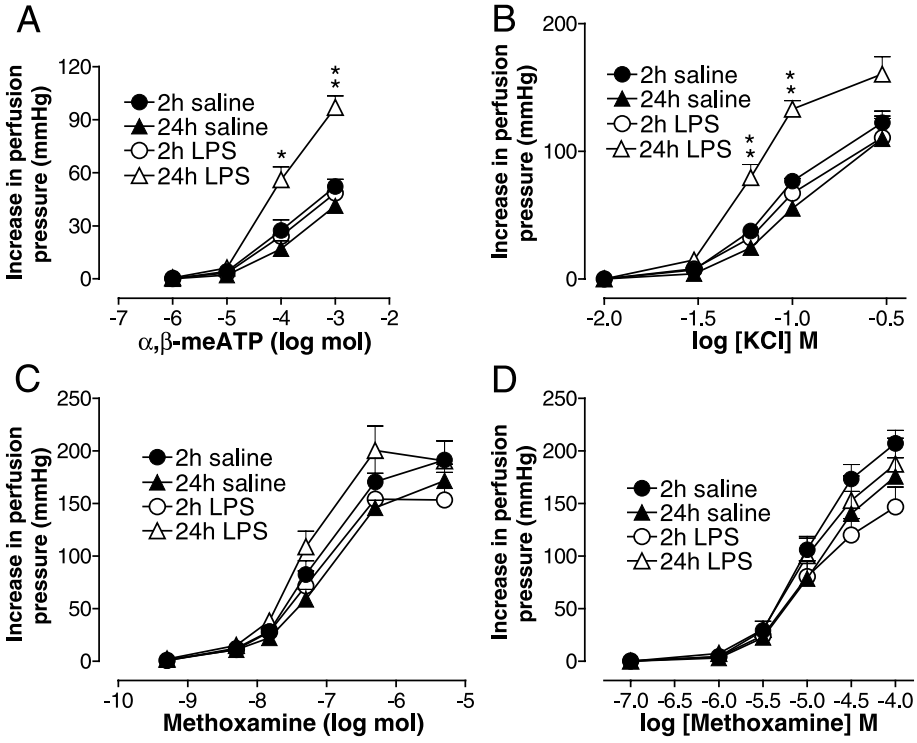

Fig. 1. Contractile responses to (A) α,β-methylene ATP doses (α,β-meATP), (B) potassium chloride (KCl) concentrations, (C) methoxamine doses, (D) methoxamine concentrations, of tail arteries isolated from rats after infusion of 2 h saline ($n=5$), 24 h saline ($n=6$), 2 h lipopolysaccharide (LPS; $n=7$) and 24 h lipopolysaccharide ($n=5$), without N^G-nitro-L-arginine methyl ester. Data shown are mean ± S.E.M. Significant difference between groups denoted by *$P<0.05$; **$P<0.01$.

had i.v. catheters implanted, 24 h before the start of i.v. infusion of saline (0.4 ml h^{-1}) or lipopolysaccharide (150 μg kg^{-1} h^{-1}; *Escherichia coli* serotype 0127:B8) (Waller et al., 1994). After either 2- or 24-h infusion, animals were anaesthetised with sodium pentobarbitone (<200 mg kg^{-1} i.v.) and killed by decapitation.

2.2. In vitro incubation with lipopolysaccharide

Segments of ventral tail arteries from male Sprague–Dawley rats (300–350 g) were isolated and cannulated for perfusion at the proximal end. Segments of thoracic aortae were also isolated and cleaned of adherent connective tissue. The tail arteries and aortae were then incubated in sterile Dulbecco's Modified Eagle's Medium for 24 h at 37 °C in an atmosphere of 95% air/5% CO_2. In all experiments, the culture medium was supplemented with 10% v v^{-1} foetal calf serum and 10% v v^{-1} streptomycin. Some of the arterial segments were incubated in the additional presence of 100 μg ml^{-1} lipopolysaccharide (O'Brien et al., 2001). Following incubation, the tail arteries and aortae were removed and placed in organ baths for perfusion and superfusion at 2 ml min^{-1} (tail arteries) and isometric recording (aortae).

2.3. Tail arteries

Segments of ventral tail arteries (about 4 cm long) were isolated and cannulated for perfusion at the proximal end. They were perfused and superfused at 2 ml min^{-1} with Krebs' solution containing (mM): NaCl 106.1, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 1.9, glucose 10. After 30 min equilibration, contractile dose–response curves to bolus doses of α,β-methylene ATP (50 pmol–0.5 μmol) and methoxamine (0.5 nmol–5 μmol), and cumulative concentration–response curves to methoxamine (0.1–100 μM) and KCl (10–300 mM) were constructed, in that order. Concentration–response curves to α,β-methylene ATP were not established because of the rapid desensitization of P2X receptors that occurs with this agent. Dose interval was determined by the time to recovery of the response (at least 5 min was allowed between responses following a return to baseline). Fifteen minutes was allowed between response curves. In another group of preparations, responses to the vasoconstrictors were investigated in the presence of N^G-nitro-L-arginine methyl ester (300 μM), an inhibitor of nitric oxide synthase, added at the start of equilibration. A third group of preparations was submaximally preconstricted with methoxamine and, once stable tone had been achieved, concentration–responses curves to acetylcholine were established. After the response curve had been completed, there was a washout period of 30 min, following which tone was reestablished by addition of methoxamine, and a concentration–response curve to sodium nitroprusside was then established. Drugs applied as doses were injected in a volume of 50 μl into a norprene injection port proximal to the preparation. Drugs applied as concentrations were added to the perfusate reservoir.

2.4. Aortae

Thoracic aortae were removed and cleaned of adherent connective tissue in preparation for the in vitro endotoxaemic studies. Following incubation for 24 h in culture medium with and without lipopolysaccharide, 3–4-mm segments of aortae were cut and mounted in organ baths with 1 g resting tension applied. Following 1 h of equilibration, cumulative concentrations of methoxamine (100 nM–1 mM) were applied. Methoxamine was then removed with several washes with Krebs' solution and basal tone restored. Following a further 1 h of equilibration, with washes every 15 min, cumulative concentrations of potassium chloride (10–300 mM) were applied.

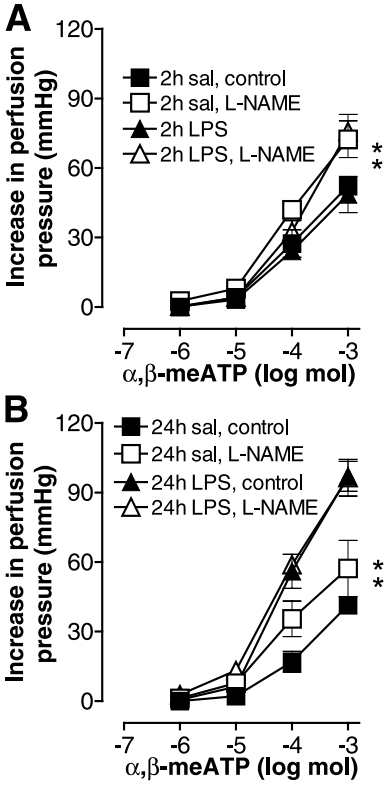

Fig. 2. Contractile responses to α,β-methylene ATP (α,β-meATP) of tail arteries isolated from rats after infusion of (A) 2 h saline (sal), 2 h lipopolysaccharide (LPS) and (B) 24 h saline, 24 h lipopolysaccharide, without and with N^G-nitro-L-arginine methyl ester (L-NAME; 300 μM). Control data are the same as in Fig. 1 ($n=5–7$). Data in the presence of N^G-nitro-L-arginine methyl ester are the means of four to eight separate experiments. Data shown are mean ± S.E.M. L-NAME significantly increased contractions in all groups except the 24-h LPS group. Significant difference within 2-h saline, 24-h saline and 2-h lipopolysaccharide groups, between curves generated in the absence and presence of L-NAME, is denoted by $^{**}P<0.01$.

2.5. Data analysis

Contractile responses were measured as increase in tone above baseline (mm Hg) (tail arteries) or increase in tension (g) (aortae). Responses curves were compared by analysis of variance with Tukey's post hoc test (GraphPad Prism). Where response curves reached a maximum, maximal responses (R_{max}) and pEC_{50} values were calculated. Vasorelaxant responses were measured as a percentage of the methoxamine-induced increase in tone above baseline; R_{max} and pEC_{50} values were calculated and compared by analysis of variance. Data are presented as means ± S.E.M. Differences were only considered significant if $P<0.05$. Data for contractile responses of the ex vivo tail arteries were analysed twice: (1) between group comparisons (in the absence and presence of N^G-nitro-L-arginine methyl ester), (2) effect of N^G-nitro-L-arginine methyl ester on response of contractile agent. Hence, Bonferroni correction was applied to bring the α level overall back to 0.05.

2.6. Drugs

All drugs and Dulbecco's Modified Eagle's Medium were obtained from Sigma. All drugs were made up in distilled water.

3. Results

3.1. Basal perfusion pressures

There were no significant differences in basal perfusion pressure between the groups, which were 21.7 ± 2.3 ($n=10$), 22.1 ± 1.5 ($n=15$), 21.9 ± 1.6 ($n=15$) and 21.4 ± 2.7 mm Hg ($n=9$), in the 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively.

3.2. Contractile responses to α,β-methylene ATP and potassium chloride

The tail arteries produced dose-dependent contractions to α,β-methylene ATP. The maximal attained response to α,β-methylene ATP (at 50 nmol) was greater after 24 h lipopolysaccharide infusion (97 ± 7 mm Hg, $n=5$) than in the 2-h saline, 24-h saline and 2-h lipopolysaccharide groups (52 ± 3 mm Hg, $n=5$; 41 ± 3 mm Hg, $n=6$; 49 ± 8 mm Hg, $n=7$, respectively) ($P<0.01$) (Fig. 1A).

Contractile responses to cumulative concentrations of potassium chloride were also greater 24 h after lipopolysaccharide infusion compared to responses in the other groups. At 0.1 M potassium chloride, responses at 24 h after lipopolysaccharide infusion (133 ± 7 mm Hg, $n=5$)

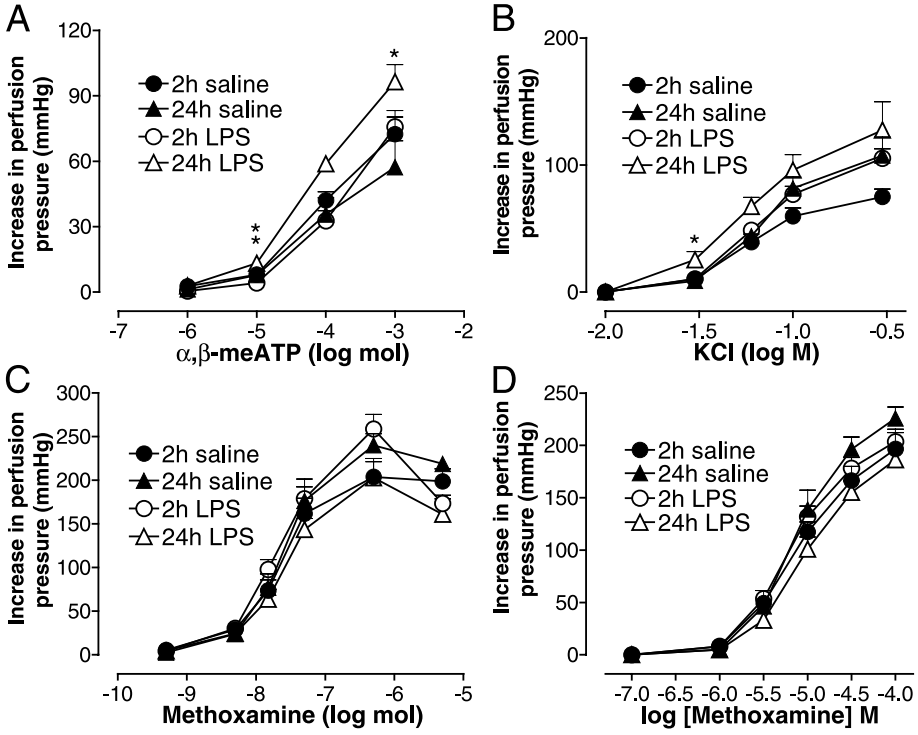

Fig. 3. Contractile responses to (A) α,β-methylene ATP doses (α,β-meATP), (B) potassium chloride (KCl) concentrations, (C) bolus methoxamine doses, (D) cumulative methoxamine concentrations, of tail arteries isolated from rats after infusion of 2 h saline ($n=4–5$), 24 h saline ($n=4$), 2 h lipopolysaccharide (LPS; $n=8$) and 24 h lipopolysaccharide ($n=5$), with N^G-nitro-L-arginine methyl ester (300 μM), i.e. data from Figs. 2, 4 and 5). Data shown are mean ± S.E.M. Significant differences between the groups in panels A–D are shown as: (A) 24 h lipopolysaccharide and 24 h saline $*P<0.05$; 24 h lipopolysaccharide and 2 h lipopolysaccharide $**P<0.01$. (B) 24 h lipopolysaccharide vs. all other groups $*P<0.05$.

were about twice the corresponding responses obtained to potassium chloride after 2 h saline, 24 h saline and 2 h lipopolysaccharide infusion (77 ± 2 mm Hg, $n = 5$; 55 ± 4 mm Hg, $n = 6$; 67 ± 12 mm Hg, $n = 7$, respectively) ($P < 0.01$) (Fig. 1B).

3.3. Contractile responses to methoxamine

The tail arteries produced dose-dependent contractions to methoxamine. There were no significant differences between the groups. R_{max} values were 191 ± 18 ($n = 5$), 171 ± 16 ($n = 6$), 153 ± 26 (n = 7) and 190 ± 19 mm Hg ($n = 5$) after 2 h saline, 24 h saline, 2 h lipopolysaccharide and 24 h lipopolysaccharide, respectively. pEC_{50} values were 7.14 ± 0.15, 7.02 ± 0.16, 7.27 ± 0.11, 7.38 ± 0.07 after 2 h saline, 24 h saline, 2 h lipopolysaccharide and 24 h lipopolysaccharide, respectively (Fig. 1C).

Concentration–response curves to methoxamine did not reach a maximum, but there was no significant difference in responses between the groups at the maximal concentration of methoxamine used (0.1 mM): these values at 2 h saline, 24 h saline, 2 h lipopolysaccharide and 24 h lipopolysaccharide were 207 ± 12 ($n = 5$), 176 ± 18 ($n = 6$), 147 ± 19 ($n = 7$) and 187 ± 25 mm Hg ($n = 5$), respectively (Fig. 1D).

3.4. Effect of N^G-nitro-L-arginine methyl ester on contractile responses to α,β-methylene ATP, methoxamine and potassium chloride

Endothelial damage or blockade of endothelial vasorelaxant mechanisms can lead to an augmentation of vasoconstrictor responses. Therefore, in order to investigate whether this was involved in the augmented responses that we observed to α,β-methylene ATP and potassium chloride, we examined responses to these agents, and to methoxamine, in the presence of N^G-nitro-L-arginine methyl ester (300 μM). This approach was used because of the possibility of smooth muscle damage, and reduced viability of the preparation, upon removal of the endothelium.

N^G-nitro-L-arginine methyl ester augmented contractions to bolus doses of α,β-methylene ATP in the 2-h saline ($n = 5$), 24-h saline ($n = 4$) and 2-h lipopolysaccharide ($n = 8$) groups ($P < 0.01$) (Fig. 2A and B). In contrast, N^G-nitro-L-arginine methyl ester had no significant effect on responses to α,β-methylene ATP in the 24-h lipopolysaccharide group ($n = 5$) (Fig. 2B). N^G-nitro-L-arginine methyl ester reduced, but did not abolish, the difference in contractility between the four groups (Fig. 3A).

N^G-nitro-L-arginine methyl ester also augmented contractions to bolus doses of methoxamine in the 2-h saline ($n = 4$), 24-h saline ($n = 4$) and 2-h lipopolysaccharide ($n = 8$) groups (Fig. 4A and B). In contrast, N^G-nitro-L-arginine methyl ester had no significant effect on responses to doses of methoxamine in the 24-h lipopolysaccharide group ($n = 5$) (Fig. 4B).

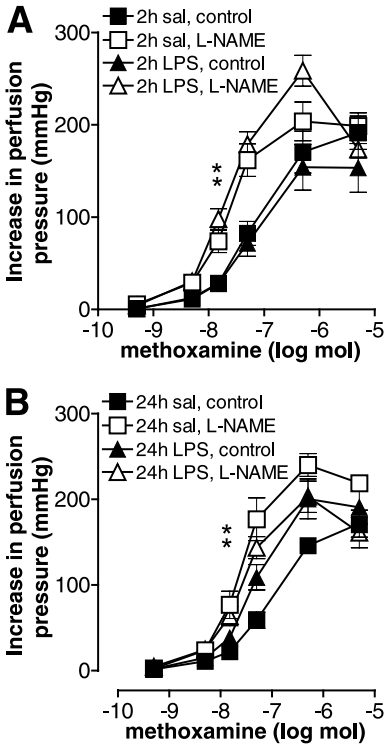

Fig. 4. Contractile responses to bolus methoxamine doses of tail arteries isolated from rats after infusion of (A) 2 h saline (sal), 2 h lipopolysaccharide (LPS) and (B) 24 h saline, 24 h lipopolysaccharide, without and with N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM). Control data are the same as in Fig. 1 ($n = 5–7$). Data in the presence of N^G-nitro-L-arginine methyl ester are the means of four to eight separate experiments. Data shown are mean ± S.E.M. L-NAME significantly increased contractions in all groups except the 24-h LPS group. Significant difference within 2-h saline, 24-h saline and 2-h lipopolysaccharide groups, between curves generated in the absence and presence of L-NAME, is denoted by $^{**}P < 0.01$.

In the presence of N^G-nitro-L-arginine methyl ester, there was no significant difference between the groups (Fig. 3C). pEC_{50} values were 7.62 ± 0.07 ($n = 4$), 7.59 ± 0.07 ($n = 4$), 7.6 ± 0.05 ($n = 8$) and 7.6 ± 0.09 ($n = 5$) in the 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively.

Contractions to cumulative concentrations of methoxamine were also augmented by N^G-nitro-L-arginine methyl ester in the 24-h saline ($n = 4$) and 2-h lipopolysaccharide ($n = 8$) groups (Fig. 5), although the increase was not as pronounced as observed with the doses of methoxamine. In contrast, N^G-nitro-L-arginine methyl ester had no significant effect on responses to concentrations of methoxamine in the 2-h saline ($n = 4$) and 24-h lipopolysaccharide ($n = 5$) groups (Fig. 5). In the presence of N^G-nitro-L-arginine methyl ester, there was no difference in concentration-dependent contractions between the four groups (Fig. 3D).

Responses to cumulative concentrations of potassium chloride were unaffected by N^G-nitro-L-arginine methyl ester in the 24-h saline and 2-h lipopolysaccharide groups,

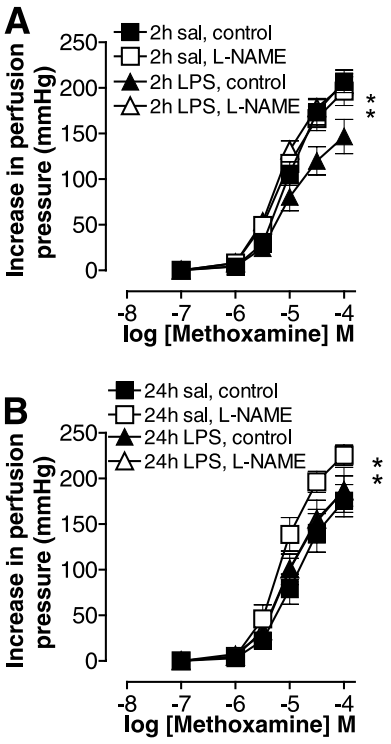

Fig. 5. Contractile responses to cumulative methoxamine concentrations of tail arteries isolated from rats after infusion of (A) 2 h saline (sal), 2 h lipopolysaccharide (LPS) and (B) 24 h saline, 24 h lipopolysaccharide, without and with N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM). Control data are the same as in Fig. 1 ($n=5-7$). Data in the presence of N^G-nitro-L-arginine methyl ester are the means of four to eight separate experiments. Data shown are mean ± S.E.M. L-NAME augmented responses in the 24-h saline and 2-h LPS groups. Significant difference within 24-h saline and 2-h LPS groups, between curves generated in the absence and presence of L-NAME, is denoted by $^{**}P<0.01$.

but responses were reduced in both the 2-h saline and 24-h lipopolysaccharide groups, compared to their respective controls in the absence of N^G-nitro-L-arginine methyl ester (Figs. 1B and 3B). In the presence of N^G-nitro-L-arginine methyl ester, responses to potassium chloride in the 24-h lipopolysaccharide group were significantly different from the other three groups only at a single concentration of 30 mM (Fig. 3B).

3.5. Vasorelaxant responses to sodium nitroprusside and acetylcholine

Maximal concentration-dependent relaxations to sodium nitroprusside were greater after 24 h lipopolysaccharide infusion than in the 2-h saline, 24-h saline and 2-h lipopolysaccharide groups (Fig. 6A). R_{max} values were 78.6 ± 3.9% ($n=5$), 83.9 ± 3.7% ($n=7$), 83.1 ± 2.1% ($n=8$) and 96.8 ± 0.8% ($n=4$) in 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively ($P<0.01$). pEC_{50} values were not significantly different between the groups: 6.63 ± 0.3 ($n=5$), 6.79 ± 0.16 ($n=7$), 6.57 ± 0.11 ($n=8$) and 7.14 ± 0.09 ($n=4$) in 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively.

Concentration-dependent relaxations to acetylcholine were not significantly different between the four groups (Fig. 6B). R_{max} values were 34.6 ± 4.0% ($n=6$), 39.0 ± 13.8% ($n=5$), 44.7 ± 8.4% ($n=8$) and 53.8 ± 11.8% ($n=4$) in 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively. pEC_{50} values were 6.83 ± 0.17 ($n=6$), 6.76 ± 0.24 ($n=5$), 6.99 ± 0.11 ($n=8$) and 6.85 ± 0.16 ($n=4$) in 2-h saline, 24-h saline, 2-h lipopolysaccharide and 24-h lipopolysaccharide groups, respectively.

3.6. Effect of 24 h in vitro incubation with lipopolysaccharide on contractile responses to methoxamine, α,β-methylene ATP and potassium chloride

In order to investigate whether the changes in vascular responsiveness that we observed at 24 h after lipopolysaccharide infusion were due to local effects of lipopolysaccharide, tail arteries were incubated for 24 h in serum with and without lipopolysaccharide. There was no significant difference between responses to α,β-methylene ATP, methoxamine and potassium chloride of tail arteries incubated for 24 h in culture medium compared to those incubated in the presence of culture medium plus lipopolysaccharide (Fig. 7).

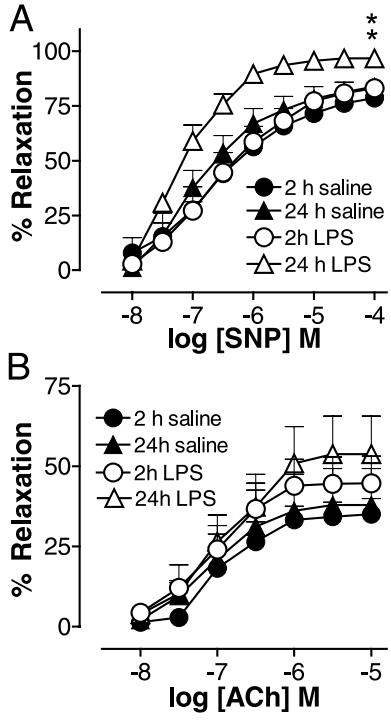

Fig. 6. Vasorelaxant responses to (A) sodium nitroprusside (SNP) and (B) acetylcholine (ACh) in tail arteries isolated from rats after infusion of 2 h saline, 24 h saline, 2 h lipopolysaccharide (LPS) and 24 h lipopolysaccharide ($n=4-8$). Data shown are mean ± S.E.M. Significant difference between maximal responses of groups denoted by $^{**}P<0.01$.

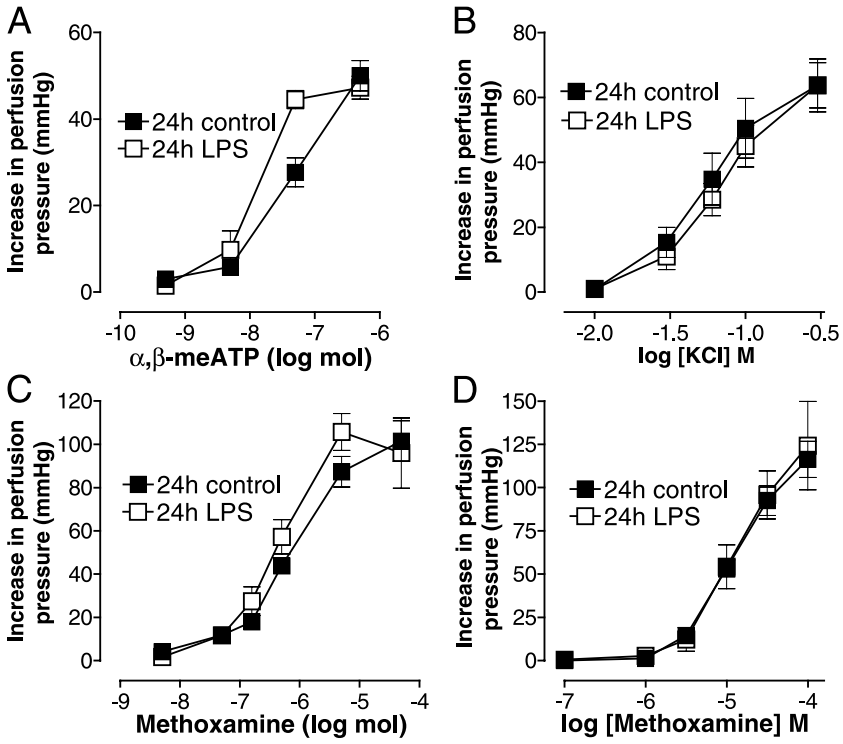

Fig. 7. Contractile responses to (A) α,β-methylene ATP (α,β-meATP), (B) potassium chloride (KCl), (C) bolus methoxamine doses, (D) cumulative methoxamine concentrations, of tail arteries isolated from rats after in vitro incubation for 24 h in culture medium (control, $n=6$) or culture medium plus lipopolysaccharide (LPS; $n=4$). Data shown are mean ± S.E.M.

Since others had shown that in vitro incubation of aortae with lipopolysaccharide produces hypocontractility to phenylephrine (Hall et al., 1996), the effectiveness of the 24-h lipopolysaccharide incubation was tested using isolated aortic rings. In aortae equilibrated for 24 h in culture medium plus lipopolysaccharide, contractions to methoxamine were virtually abolished: maximal contractions to methoxamine (at 1 mM) after 24 h incubation in culture medium plus lipopolysaccharide at 0.0 ± 0.03 g ($n=5$) were impaired compared to contractions elicited after 24 h incubation of aortae in culture medium without lipopolysaccharide (0.15 ± 0.05 g, $n=5$) ($P<0.01$). There was no significant difference between contractile responses to potassium chloride of aortae incubated in culture medium (0.83 ± 0.24 g, $n=3$) and culture medium plus lipopolysaccharide (0.51 ± 0.16 g, $n=3$).

4. Discussion

The present study has shown that tail arteries isolated from rats after infusion of lipopolysaccharide for 24 h display an augmented contractile responsiveness to α,β-methylene ATP and potassium chloride. Thus, the tail artery is clearly different to most other blood vessels which, if affected by endotoxin, generally show an impaired contractile responsiveness. Two different lines of evidence indicate that the hypercontractility that we have observed may involve, at least in part, an impaired endothelial production and/or release of nitric oxide at 24 h after lipopolysaccharide infusion. Firstly, N^G-nitro-L-arginine methyl ester failed to augment contractile responses in tail arteries from the 24-h lipopolysaccharide group, and secondly, vasorelaxant responses to sodium nitroprusside were augmented in tail arteries from the 24-h lipopolysaccharide group.

An increase in contractile responsiveness to α,β-methylene ATP and potassium chloride, but not to methoxamine, indicates that there is heterogeneity of changes in responses to different vasoconstrictors in the tail artery, in endotoxaemia induced by in vivo lipopolysaccharide infusion in rats. We have previously shown within-tissue differences in responses to vasoconstrictors in this model of endotoxaemia, as aortae of rats taken at 24 h after lipopolysaccharide treatment were hyporesponsive to methoxamine, but showed no changes in responses to the thromboxane A_2 agonist U46619 (Farmer et al., 2001). Heterogeneity of changes in responses to different vasoconstrictors has also recently been reported in rat superior mesenteric artery in an in vitro model of endotoxaemia (O'Brien et al., 2001). The mechanisms underlying these differences are complex and not fully understood. They may involve the different intracellular signalling pathways of the vasoconstrictors as well as the ability of external factors to modulate the response. Between-tissue differences in changes in contractile responses were also evident, as the lack of hypocontractility to vasoconstrictors in the tail artery is clearly different to the

hypocontractility displayed by other blood vessels (mesenteric bed, aorta) isolated from rats in this model of endotoxaemia (Farmer et al., 2001, 2002).

It is known that endothelial denudation, or blockade of endothelial vasorelaxant mechanisms, particularly nitric oxide, can lead to augmented responses to vasoconstrictors (Cederqvist et al., 1991; Bucher et al., 1992; Li and Duckles, 1992). Thus, our data indicate that one mechanism that may be involved in the increased response to α,β-methylene ATP in tail arteries at 24 h after lipopolysaccharide infusion is an impaired production and/or release of nitric oxide. This is because N^G-nitro-L-arginine methyl ester was unable to augment contractile responses to α,β-methylene ATP in this group of tail arteries, although it clearly increased responses to α,β-methylene ATP in the 2-h and 24-h saline groups, as well as in the 2-h lipopolysaccharide group. N^G-nitro-L-arginine methyl ester also failed to augment contractile responses to doses of methoxamine in tail arteries from rats infused with lipopolysaccharide for 24 h but, in contrast, it increased these responses in tail arteries isolated from the other three groups of rats. A similar pattern of change was observed for the effects of N^G-nitro-L-arginine methyl ester on responses to concentrations of methoxamine.

If there was endothelial damage, it is unclear why there was no increase in the contractile response to methoxamine in tail arteries from the 24-h lipopolysaccharide group under control conditions. As we have consistently observed an impaired contractile response to methoxamine in mesenteric arterial beds (Farmer et al., 2001), aortae (Farmer et al., 2002) and renal arterial beds (unpublished observations) taken from this model of endotoxaemia, one possibility is that an impaired contractility to methoxamine also occurs in tail arteries, and this opposes the increase in smooth muscle contractility. Indeed, this could be a consequence of α-adrenoceptor downregulation due to the elevated circulating levels of catecholamines that are known to occur in endotoxaemia (Roth and Spitzer, 1987; Jones et al., 1988).

Further suggestion of possible damage to the endothelium came from the observation that relaxations to sodium nitroprusside were augmented in tail arteries at 24 h after lipopolysaccharide infusion, but not in any of the other groups. Endothelial denudation, or block of nitric oxide synthase, is known to cause an increase in smooth muscle vasorelaxation to nitrovasodilators, which is believed to be due to an increase in the available pool of guanylate cyclase/cGMP for activation, following relief from activation by tonically produced nitric oxide (Busse et al., 1989; Luscher et al., 1989; Jackson and Busse, 1991; Ralevic et al., 1991; Toyoshima et al., 1998). Although damage to the endothelium would be predicted to cause an impairment of responses to acetylcholine (Gerkens, 1988), these responses were not significantly different between the groups, suggesting that any decrease in endothelium-dependent vasorelaxation may have been opposed by the increase in smooth muscle vasorelaxation.

N^G-nitro-L-arginine methyl ester failed to normalise responses to α,β-methylene ATP and potassium chloride between the groups; indeed, it did not augment the contractile responses to potassium chloride, indicating that the endothelium/nitric oxide is not the sole mechanism by which there is an increase in contractile responses in tail arteries at 24 h following lipopolysaccharide infusion. Alterations in the smooth muscle contractile apparatus of the tail artery at 24 h after lipopolysaccharide infusion are implicated, but the precise nature of these changes remains to be determined. The potassium chloride response curve was the last to be carried out in our experimental protocol, and it is possible that with the construction of each preceding contractile response curve, there was progressive endothelial damage due to shear stress, leaving little endothelial nitric oxide to be blocked by N^G-nitro-L-arginine methyl ester. Indeed, augmentation by N^G-nitro-L-arginine methyl ester was generally greater for the dose–response curves to methoxamine than for the subsequently constructed methoxamine concentration–response curves, suggesting that there may have been some damage to the endothelium with time. On the other hand, inhibitory effects of the endothelium on vasocontractile responses are agonist-specific, and some investigators have shown a lack of effect of endothelium removal on contractions to potassium chloride, whilst contractions to other agents are enhanced (Oshiro et al., 1985; Connor and Feniuk, 1989). Furthermore, depolarising concentrations of KCl will release neurotransmitters from the nerve terminals, which may add to the complexity of the response. We have no ready explanation as to why responses to the maximal concentration of potassium chloride in the presence of N^G-nitro-L-arginine methyl ester in the 2-h saline and 24-h lipopolysaccharide groups were reduced, but it could be that the responses were more variable at the end of the experimental protocol.

In order to determine whether the vascular effects of lipopolysaccharide treatment were induced locally, tail arteries were incubated with culture medium with and without lipopolysaccharide in vitro for 24 h. The lipopolysaccharide treatment had no significant effect on responses to the contractile agents, indicating a possible requirement for additional factors/mechanisms present in vivo to induce the changes in arterial function that we observed. The success of the in vitro lipopolysaccharide protocol was confirmed in aortic rings, in which methoxamine-induced contractions were attenuated. This is consistent with previous studies which showed that in vitro incubation of rat isolated aortae and mesenteric arteries with lipopolysaccharide causes an impaired adrenoceptor-mediated contractility (Julou-Schaeffer et al., 1990; McKenna, 1990; Hall et al., 1996; O'Brien et al., 2001). In contrast, in vitro incubation with endotoxin failed to alter the contractile response to phenylephrine in the ovine digital artery (Pawson et al., 2000). These data provide further evidence for region-specific changes in blood vessel function in endotoxaemia both in vitro and in vivo.

In conclusion, the present study indicates maintained postjunctional mechanisms of sympathetic vasoconstriction in the rat tail artery in endotoxaemia, and others have reported a prejunctional mechanism that may preserve sympathetic vasoconstriction in endotoxaemia (Ohlmann et al., 2000). Tail arteries isolated from rats following infusion of lipopolysaccharide for 24 h show an increased contractile responsiveness to α,β-methylene ATP and potassium chloride which may involve a decrease in the release/production of nitric oxide from the endothelium. The unimpaired contractility that was observed to methoxamine in rat tail arteries after 24 h lipopolysaccharide infusion is in contrast to our previous observations in the rat isolated aorta and mesenteric arterial bed (Farmer et al., 2001, 2002), indicating that there is heterogeneity of changes in responses to vasoconstrictors in different blood vessels in endotoxaemia.

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