British Journal of Pharmacology (1998) 125, 1543–1550
http://www.stockton-press.co.uk/bjp

The influence of antibodies to TNF-α and IL-1β on haemodynamic responses to the cytokines, and to lipopolysaccharide, in conscious rats

[1,3]S.M. Gardiner, [1]P.A. Kemp, [1]J.E. March, [2]J. Woolley & [1]T. Bennett

[1]School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [2]Therapeutic Antibodies, Charterhouse Square, London

1 Male, Long Evans rats (350–450 g) were anaesthetized and had pulsed Doppler probes and intravascular catheters implanted to allow monitoring of regional (renal, mesenteric and hindquarters) haemodynamics in the conscious state. Our main objectives were to:- assess the effects of administering human recombinant tumour necrosis factor (TNF)-α and human recombinant interleukin-1 (IL-1)β, alone and together; determine the influence of pretreatment with a mixture of antibodies to TNF-α and IL-1β on responses to co-administration of the cytokines; ascertain if pretreatment with a mixture of the antibodies to TNF-α and IL-1β had any influence on the responses to lipopolysaccharide (LPS).

2 TNF-α (10, 100 and 250 μg kg^{-1}, in separate groups, $n=3$, 9 and 8, respectively) caused tachycardia (maximum Δ, $+101\pm9$ beats min^{-1}) and modest hypotension (maximum Δ, -10 ± 2 mmHg), accompanied by variable changes in renal and mesenteric vascular conductance, but clear increases in hindquarters vascular conductance; only the latter were dose-related (maximum Δ, $+6\pm6$, $+27\pm9$, and $+61\pm12$% at 10, 100 and 250 μg kg^{-1}, respectively).

3 IL-1β (1, 10, and 100 μg kg^{-1} in separate groups, $n=8$, 8 and 9, respectively) evoked changes similar to those of TNF-α (maximum Δ heart rate, $+69\pm15$ beats min^{-1}; maximum Δ mean blood pressure, -14 ± 2 mmHg; maximum Δ hindquarters vascular conductance, $+49\pm17$%), but with no clear dose-dependency.

4 TNF-α (250 μg kg^{-1}) and IL-1β (10 μg kg^{-1}) together caused tachycardia (maximum Δ, $+76\pm15$ beats min^{-1}) and hypotension (maximum Δ, -24 ± 2 mmHg) accompanied by increases in renal, mesenteric and hindquarters vascular conductances ($+52\pm6$%, $+23\pm8$%, and $+52\pm11$%, respectively). Thereafter, blood pressure recovered, in association with marked reductions in mesenteric and hindquarters vascular conductances (maximum Δ, -50 ± 3% and -58 ± 3%, respectively). Although bolus injection of LPS (3.5 mg kg^{-1}) caused an initial hypotension (maximum Δ, -27 ± 11 mmHg) similar to that seen with co-administration of the cytokines, it did not cause mesenteric or hindquarters vasodilatation, and there was only a slow onset renal vasodilatation. The recovery in blood pressure following LPS was less than after the cytokines, and in the former condition there was no mesenteric vasoconstriction. By 24 h after co-administration of TNF-α and IL-1β or after bolus injection of LPS, the secondary reduction in blood pressure was similar (-16 ± 2 and -13 ± 3 mmHg, respectively), but in the former group the tachycardia ($+117\pm14$ beats min^{-1}) and increase in hindquarters vascular conductance ($+99\pm21$%) were greater than after bolus injection of LPS ($+54\pm16$ beats min^{-1} and $+43\pm9$%, respectively).

5 Pretreatment with antibodies to TNF-α and IL-1β (300 mg kg^{-1}) blocked the initial hypotensive and mesenteric and hindquarters vasodilator responses to co-administration of the cytokines subsequently. However, tachycardia and renal vasodilatation were still apparent. Premixing antibodies and cytokines before administration prevented most of the effects of the latter, but tachycardia was still present at 24 h.

6 Pretreatment with antibodies to TNF-α and IL-1β before infusion of LPS (150 μg kg^{-1} h^{-1} for 24 h) did not affect the initial fall in blood pressure, but suppressed the hindquarters vasodilatation and caused a slight improvement in the recovery of blood pressure. However, pretreatment with the antibodies had no effect on the subsequent cardiovascular sequelae of LPS infusion.

7 The results indicate that although co-administration of TNF-α and IL-1β can evoke cardiovascular responses which, in some respects, mimic those of LPS, and although antibodies to the cytokines can suppress most of the cardiovascular effects of the cytokines, the antibodies have little influence on the haemodynamic responses to LPS, possibly because, during infusion of LPS, the sites of production and local action of endogenous cytokines, are not accessible to exogenous antibodies.

Keywords: Tumour necrosis factor-α; interleukin-1β; lipopolysaccharide; antibodies

Abbreviations: LPS, lipopolysaccharide; RVC/MVC/HVC, renal/mesenteric/hindquarters vascular conductances, respectively

[3] Author for correspondence.

Introduction

Cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) have been implicated in the cardiovascular sequelae of endotoxaemia (see Cavaillon *et al*., 1992; Parrillo, 1993; Blackwell & Christman, 1996; Galley & Webster, 1996, for reviews). However, their detailed cardiovascular effects *in vivo* have not been well characterized and, particularly, it is not known if co-infusion of TNF-α and IL-1β produces cardiovascular changes similar to those seen with lipopolysaccharide (LPS) administration. Therefore, the first objective of the present work was to determine any dose-response relationships for the regional haemodynamic effects of TNF-α and of IL-1β in conscious, unrestrained rats. Subsequently, we compared the effects of co-administration of the cytokines to those seen after a bolus injection of LPS.

Although, in theory, antibodies to cytokines might be of benefit in endotoxaemia, clinical evidence for this is generally lacking (see Parillo, 1993; Bernard, 1995; Baue, 1996; Galley & Webster, 1996; Deitch, 1998, for reviews). Moreover, in experimental animals, antibodies to TNF-α, for example, do not always affect the cardiovascular responses to LPS administration (e.g., Waller *et al*., 1995; Xie *et al*., 1997). However, we are not aware of any *in vivo* assessment of the effects of antibodies to TNF-α and IL-1β on the responses to co-administration of the cytokines themselves, as a baseline against which to judge any possible effect of a mixture of the antibodies on responses to LPS. This is an important question because there is evidence that anti-cytokine antibodies can act as carrier proteins, thereby prolonging, rather than suppressing, the actions of the cytokines (Finkelmann *et al*., 1993). Therefore, the second objective was to determine the extent to which treatment with different doses of a mixture of antibodies to TNF-α and IL-1β affected the cardiovascular responses to a mixture of the cytokines.

Having delineated the influence of a mixture of antibodies to TNF-α and IL-1β on the cardiovascular effects of the cytokines, we could then address the third aim of the study, which was, by determining the ability of the antibody mixture to influence the cardiovascular effects of LPS infusion in conscious rats, to test the hypothesis that these cytokines are responsible for the cardiovascular events seen in this model of endotoxaemia (Gardiner *et al*., 1995). We have shown that with continuous infusion of LPS for 24 h, as here, there is a peak in plasma TNF-α levels after 1–2 h, with levels returning to baseline by 3 h (Waller *et al*., 1995). In the same model we have not detected any change in plasma IL-1 levels (Gardiner *et al*., unpublished observations) but since plasma levels do not straightforwardly reflect tissue levels, due to binding and/or catabolism of the cytokines, that does not necessarily mean increased production of IL-1 does not contribute to the cardiovascular sequelae of LPS infusion. For this reason we considered that any functional involvement of the cytokines, either directly or indirectly, in the cardiovascular responses to LPS infusion would be more likely detected through an effect of the antibodies on the haemodynamic changes, than it would by measurements of changes in the plasma levels of cytokines.

Some of the results herein have been published in abstract form (Gardiner *et al*., 1997a,b), and presented to the British Pharmacological Society (Gardiner 1998a,b).

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham. Animals were chronically instrumented with pulsed Doppler flow probes and intravascular catheters to allow i.v. administration of substances, and the monitoring of renal, mesenteric and hindquarters haemodynamics together with systemic arterial blood pressure and heart rate (Gardiner *et al*., 1996c). All surgery was carried out under sodium methohexitone anaesthesia (Brietal Lilly, 40–60 mg kg^{-1}, i.p., supplemented as required); the flow probes were implanted at least 2 weeks before the catheters, and the latter were implanted at least 1 day before experiments were begun. The following experiments were undertaken.

Effects of TNF-α or IL-1β alone

TNF-α at doses of 10 ($n=3$), 100 ($n=9$), or 250 ($n=8$) μg kg^{-1} was administered by i.v. infusion over 45 min (2 μl min^{-1}), and recordings were made continuously during the infusion period and each hour thereafter for 8 h, with a final recording made 24 h after the onset of infusion.

A similar protocol was followed with IL-1β at doses of 1 ($n=8$), 10 ($n=8$) and 100 ($n=9$) μg kg^{-1}. A control group of rats ($n=9$) was infused with saline (2 μl min^{-1}) over 45 min, and monitored as above.

Effects of TNF-α and IL-1β co-administration, compared to LPS

TNF-α (250 μg kg^{-1}) and IL-1β (10 μg kg^{-1}) were infused (both at 2 μl min^{-1}) simultaneously *via* two separate venous catheters over 45 min in nine rats, and recordings were made as above. The doses of the cytokines were selected on the basis of their initial hypotensive effects (see Results), and the finding from pilot experiments that, together, their early effect on mean arterial pressure (MAP) matched that of a bolus injection of LPS. Prior to administration of the cytokines, this group of animals was given a bolus injection of saline to control for antibody administration.

The model of endotoxaemia we have worked with involves continuous i.v. infusion of a relatively low dose of LPS (Gardiner *et al*., 1995), and this produces relatively small changes in MAP. However, we found that bolus i.v. injection (in 0.3 ml) of LPS at a dose of 3.5 mg kg^{-1} (i.e., the amount that we would normally infuse over 24 h at 150 μg kg^{-1} h^{-1}; Gardiner *et al*., 1995) produced early changes in MAP which matched those seen with cytokine co-infusion, so a group ($n=9$) of animals was given this bolus dose of LPS, over 10 s.

Effects of antibodies to TNF-α and IL-1β

A mixture of sheep polyclonal antibodies to human recombinant TNF-α and IL-1β (Ruetten *et al*., 1996) was administered as a bolus dose (3 mg kg^{-1}, i.v.) to nine rats 1 h before the mixture of TNF-α and IL-1β. Since this dose of the antibody mixture had little effect on the responses to the cytokines (see Results), the experiment was repeated, but with the antibody mixture at a dose of 300 mg kg^{-1} ($n=8$), which necessitated it being given as an infusion (60 μl min^{-1}) over the 45 min before the cytokine mixture. To control for the effects of the antibody mixture in the absence of the cytokines, animals ($n=9$) were given the antibody mixture (300 mg kg^{-1}) by infusion beginning 1 h before infusion of saline (4 μl min^{-1}) over 45 min; recordings were then made as above.

Although pretreatment with the antibody mixture at a dose of 300 mg kg^{-1} reduced substantially the effects of the

cytokine mixture given subsequently, not all cardiovascular changes were abolished (see Results). A likely explanation of this observation is that, *in vivo*, the antibody mixture was unable to bind all the TNF-α and IL-1β administered before the cytokines interacted with their receptors. Therefore, we carried out a further experiment ($n=8$) to determine if premixing the cytokines with the antibodies (300 mg kg^{-1}) before administration was able to prevent all cardiovascular changes. The antibodies (in 2.5 ml) were mixed with the cytokines (in 0.2 ml), and the total volume was infused over 45 min. As a volume control for this protocol, saline (2.5 ml) was premixed with the cytokine mixture (0.2 ml) and this was infused over 45 min ($n=8$).

Finally, groups ($n=8$ in each) of rats were given the antibody mixture at 3 or 300 mg kg^{-1} starting 1 h before the onset of LPS infusion (150 μg kg^{-1} h^{-1} at 0.4 ml h^{-1}, i.v.) for 24 h. As a control, a group of animals ($n=9$) was given a 24 h infusion of LPS (as above) after saline administration.

Data analysis

Within-group analysis of data was by Friedman's test (Theodorsson-Norheim, 1987), and between-group analysis was by the Mann-Whitney *U*-test or the Kruskal-Wallis test, as appropriate. A P value <0.05 was taken as significant.

Cytokines, antibodies and LPS

Recombinant human TNF-α was purchased from R & D Systems (Minneapolis, U.S.A.), and recombinant human IL-1β from Peprotech (Rocky Hill, U.S.A.). For the haemodynamic studies the cytokines were diluted with sterile saline and frozen in aliquots prior to use. The TNF-α and IL-1β antibodies were raised in sheep against human recombinant TNF-α and IL-1β. Sheep were immunized monthly with 80 μg each of TNF-α and IL-1β in Freund's complete adjuvant in the primary immunization, and in the incomplete adjuvant for all subsequent immunizations. Serum was obtained by centrifugation of the clotted blood and subjected to sodium sulphate fractionation (18% wv^{-1}). The IgG fraction was then digested to the fab fragments using solid phase papain, and the fab so produced subjected to ion exchange chromatography. The purified material was then freeze-dried, and was dissolved in sterile saline prior to administration. LPS (*E. coli* serotype 0127:B8) was purchased from Sigma (U.K.) and dissolved in sterile saline.

Results

Effects of TNF-α or IL-1β alone

In this section, for clarity, the time points when any changes were significant ($P<0.05$) are indicated in parentheses in the text and not on the figures. The times given are from when the cytokine administration started.

During infusion of saline, heart rate (HR) fell slightly (at 0.25 and 2–9 h) as did mean arterial blood pressure (MAP; at 0.25–2 and 9 and 24 h) (Figure 1). Mesenteric vascular conductance (MVC) decreased (at 1, 3–7 and 9 h), but renal vascular conductance (RVC) and hindquarters vascular conductance (HVC) did not change significantly (Figure 1).

TNF-α at 10 μg kg^{-1} caused tachycardia (at 0.25 and 3–4 h) and a fall in MAP (at 0.25, 1 and 5 h) (Figure 1). There were reductions in MVC (at 0.75, 1 and 6–8 h) and HVC (at 0.25, 0.5, 3 and 6–24 h), but no change in RVC (Figure 1).

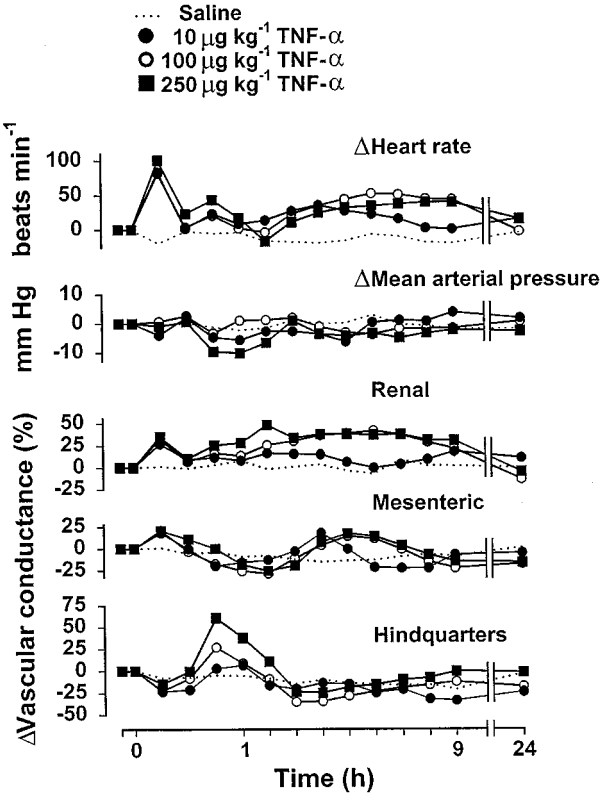

Figure 1 Cardiovascular changes in conscious, Long Evans rats, during and after administration (over 45 min) of saline ($n=9$) or TNF-α (10 μg kg^{-1}, $n=3$; 100 μg kg^{-1}, $n=9$, or 250 μg kg^{-1}, $n=8$), starting at time = 0. Note the variation in intervals on the time axis. For clarity, statistics are given in the text, and s.e.means omitted; values shown are means.

TNF-α at 100 μg kg^{-1} caused an increase in HR (at 0.25, 0.75 and 3–9 h) and a fall in MAP (at 0.75 and 5–6 h) (Figure 1). There was an increase in RVC (at 0.25 and 0.75–9 h); MVC showed an increase (at 0.25 h), followed by a decrease (2–3 h), and a subsequent secondary rise (5 and 6 h) (Figure 1); HVC initially fell (at 0.25 h), then rose (at 0.75 h), and then fell again (3–6 h) (Figure 1).

TNF-α at 250 μg kg^{-1} caused tachycardia (at 0.25–0.75 and 3–9 h) together with a reduction in MAP (at 0.75–2 and 5–8 h). There was an increase in RVC (at 0.25 and 0.75–8 h). MVC rose initially (at 0.25 h), fell thereafter (at 1–3 h), and then rose again (4–6 h). HVC fell transiently (at 0.25 h), then rose (at 0.75–1 h) and then fell again (at 3–6 h) (Figure 1). Although TNF-α at 250 μg kg^{-1} caused the greatest tachycardia, fall in MAP and rise in HVC, with the exception of the latter, none of the cardiovascular effects of this cytokine showed a clear dose-dependency (Figure 1).

IL-1β at 1 μg kg^{-1} caused an increase in HR (at 0.25–1 and 4–7 h) and a decrease in MAP (at 0.75–2 h) (Figure 2). RVC increased (0.25–2 h), whereas MVC fell (2–9 h), and HVC showed a fall (at 0.25 h), followed by a rise (0.75–1 h).

IL-1β at 10 μg kg^{-1} evoked a tachycardia (at 0.25–1 and 4–24 h) and a fall in MAP (at 0.75–2 and 6–9 h) (Figure 2). RVC rose (at 0.25–6 h) and then fell (at 8–9 h) (Figure 2); MVC increased (at 0.25–0.5 h). HVC fell (at 0.25 h) and then increased (at 0.75–1 h) (Figure 2).

IL-1β at 100 μg kg^{-1}, caused an increase in HR (at 0.25, 0.75–1 and 4–24 h) and a fall in MAP (at 0.75–2 and 5–

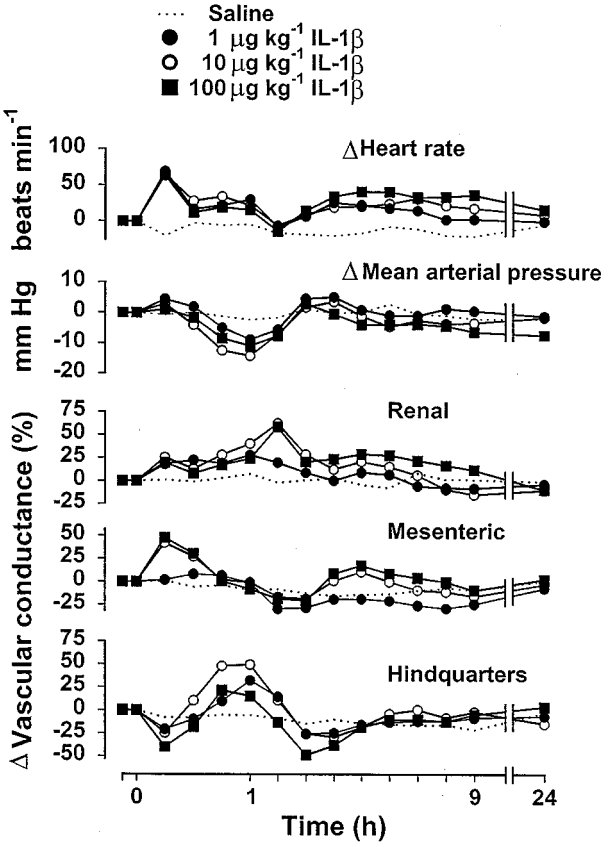

Figure 2 Cardiovascular changes in separate groups of conscious, Long Evans rats, during and after administration (over 45 min) of saline ($n=9$) or IL-1β (1 μg kg^{-1}, $n=8$, 10 μg kg^{-1}, $n=8$ or 100 μg kg^{-1}, $n=9$. Note the variation in intervals on the time axis. For clarity, statistics are given in the text, and s.e.means omitted; values shown are means.

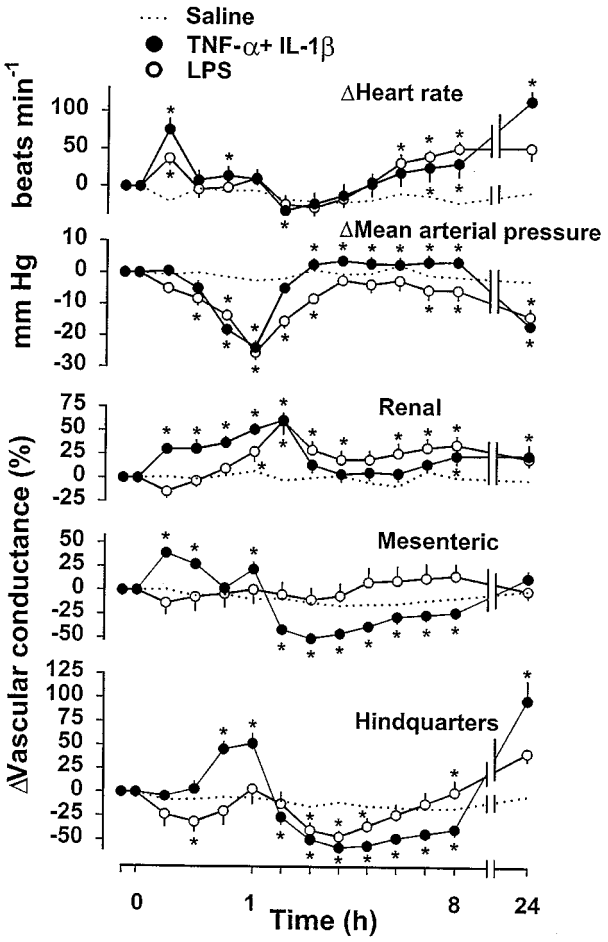

Figure 3 Cardiovascular changes in separate groups of conscious, Long Evans rats, during and after administration (over 45 min) of saline ($n=9$) or TNF-α (250 μg kg^{-1}) plus IL-1β (10 μg kg^{-1}, $n=9$) or bolus injection of lipopolysaccharide (LPS, 3.5 mg kg^{-1}, $n=9$) in 0.3 ml over 15 s, starting at time = 0. Values shown are means, and for the groups receiving cytokines or LPS, the vertical bars show s.e.mean. *$P<0.05$ versus original baseline (Friedman's test). Note the variation in the time scale.

24 h) (Figure 2). There was an increase in RVC (at 0.25 and 1–9 h) and MVC (at 0.25–0.5 and 4–8 h) (Figure 2). HVC fell (at 0.25–0.5 h), rose (at 0.75 h) and then fell again (at 3–5 h) (Figure 2). None of the effects of IL-1β showed clear dose-dependency (Figure 2).

Effects of co-administration of TNF-α and IL-1β compared to LPS

On the basis of the effects of TNF-α and IL-1β we examined the responses to a co-infusion of TNF-α (250 μg kg^{-1}) and IL-1β (10 μg kg^{-1}). The cytokine mixture caused an initial fall in MAP, tachycardia and increases in RVC, MVC and HVC (Figure 3). Thereafter, MAP recovered (at 3–8 h) the tachycardia and increase in RVC waned, and MVC and HVC were reduced (Figure 3). Between 8–24 h, MAP fell again and HR increased, together with rises in RVC and, particularly, HVC, and a fading of the reduction in MVC (Figure 3).

A bolus injection of LPS caused an initial fall in MAP similar to that seen with the co-infusion of TNF-α and IL-1β, but a slower increase in RVC, and no initial rise in MVC or HVC (Figure 3). Between 2–8 h, MAP recovered less after bolus injection of LPS than after co-administration of the cytokines, and the reduction in HVC was less marked after LPS and there was no reduction in MVC (Figure 3). By 24 h after bolus injection of LPS the increases in HR and HVC were significantly less than after co-administration of cytokines, but the other changes were not different between the groups (Figure 3).

Effects of antibodies to TNF-α and IL-1β

Administration of the antibody mixture at 3 mg kg^{-1}, starting 1 h before cytokine co-administration (as above), had no influence on the responses to the latter (data not shown).

After administration of the antibody mixture (300 mg kg^{-1}) before saline infusion, there were slight reductions in HR, RVC, MVC and HVC, but no change in MAP (Figure 4).

Pretreatment with the antibody mixture at 300 mg kg^{-1} inhibited the hypotension and the increases in MVC and HVC caused by co-administration of TNF-α and IL-1β (Figure 4); however, a tachycardia and an increase in RVC still occurred. Although the rise in RVC at 2 h was reduced (Figure 4), there was a progressive renal vasodilatation such that, by 24 h, RVC was higher than in the group receiving the cytokine mixture without antibody pretreatment (Figure 4).

Premixing the antibodies (300 mg kg^{-1}) and cytokines before administration abolished most of the effects of the cytokines, except for the rise in HR at 24 h (Figure 5).

Pretreatment with the antibody mixture at 3 mg kg^{-1} had no effect on the responses to LPS infusion (data not shown).

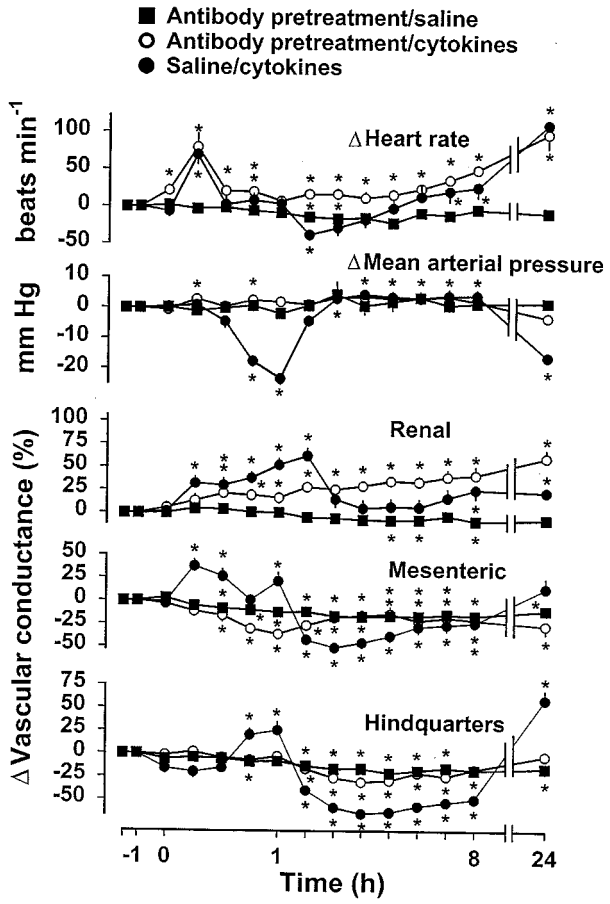

Figure 4 Cardiovascular changes in conscious Long Evans rats pretreated with a mixture of antibodies to TNF-α and IL-1β (300 mg kg^{-1} administered over 45 min) starting 1 h before administration (over 45 min) of TNF-α (250 μg kg^{-1}) plus IL-1β (10 μg kg^{-1}) ($n=8$) or saline ($n=9$). For comparison, data from the group of animals administered TNF-α (250 μg kg^{-1}) plus IL-1β (10 μg kg^{-1}) 1 h after pretreatment with saline (see Figure 3) have been plotted again. The vertical bars show s.e.mean; $^*P<0.05$ versus original baseline (Friedman's test). Note the variation in the time scale.

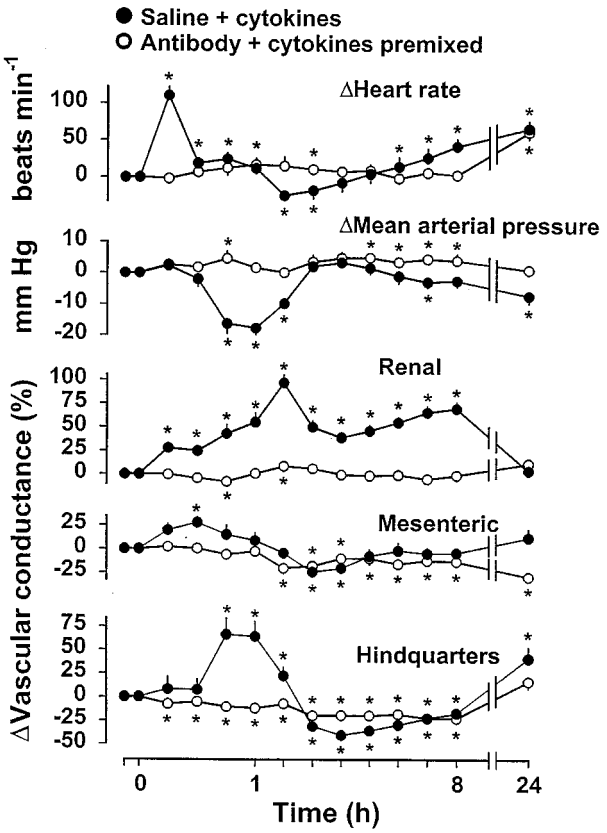

Figure 5 Cardiovascular changes in conscious Long Evans rats during and after administration (over 45 min) of saline (2.5 ml; $n=8$) or antibodies to TNF-α and IL-1β (300 mg kg^{-1}; $n=8$) premixed with TNF-α (250 μg kg^{-1}) plus IL-1β (10 μg kg^{-1}). The vertical bars show s.e.mean; $^*P<0.05$ versus original baseline (Friedman's test). Note the variation in the time scale.

Pretreatment with the antibody mixture at 300 mg kg^{-1} before infusion of LPS did not affect the initial fall in MAP, or the increases in HR or RVC, but there was a reduction in the rise in HVC (Figure 6). The attenuated increase in HVC was associated with a quicker recovery of MAP (Figure 6), but thereafter antibody pretreatment had no significant influence on the cardiovascular effects of LPS infusion (Figure 6).

Discussion

The results of the present study have shown that the cardiovascular effects of TNF-α and IL-1β are not dose-dependent, and not simply additive. Furthermore, although co-administration of TNF-α and IL-1β produces a reasonable simulation of the effects of bolus administration of LPS on MAP and HR, the associated regional haemodynamic effects differ. Additionally, we have demonstrated that pretreating animals with antibodies to TNF-α and IL-1β prevents most, but not all, of the cardiovascular effects of cytokine co-administration. However, pretreatment with antibodies to TNF-α and IL-1β before infusion of LPS suppresses the early rise in HVC and promotes the recovery from the fall in MAP, but has no other effects on the responses to LPS.

Our findings on the cardiovascular actions of TNF-α indicate that, at a dose of 250 μg kg^{-1}, it evokes significant, but modest, hypotension accompanied by clear tachycardia, and dilatation in the renal and, particularly, hindquarters vascular beds. A hypotensive, vasodilator effect of TNF-α is consistent with some observations (e.g. Takahashi *et al*., 1992; Foulkes & Shaw, 1992; Baudry *et al*., 1996), but not with others (e.g., Neilson *et al*., 1989; Turner *et al*., 1989; Morimoto *et al*., 1992; Wang *et al*., 1994; Xie *et al*., 1997), although the consensus has been that TNF-α is responsible for many of the cardiovascular sequelae of septic shock (see Tracey & Cerami, 1994, for review). From our results it is clear that an apparent lack of an effect of TNF-α on MAP (Morimoto *et al*., 1992) could result from its multifactorial actions (see Tracey & Cerami, 1994; Bazzoni & Beutler, 1996) resulting in stimulation of opposing vasodilator and vasoconstrictor influences. In this context, the ability of TNF-α to induce renal and hindquarters vasodilatation, in the absence of clear mesenteric vasodilatation is notable because TNF-α stimulates endothelin production (see Goto *et al*., 1996, for review), and elsewhere we have shown that endogenous endothelin causes particularly marked mesenteric vasoconstriction (Gardiner *et al*., 1996a,b and c).

Although the cardiovascular actions of IL-1β are less contentious than those of TNF-α, inasmuch as there is agreement that IL-1β does have cardiovascular effects, some have found IL-1β has pressor actions (e.g., Morimoto *et al*., 1992; Bataillard & Sassard, 1994; Watanabe *et al*., 1996), while others have shown convincing depressor effects (see Dinarello,

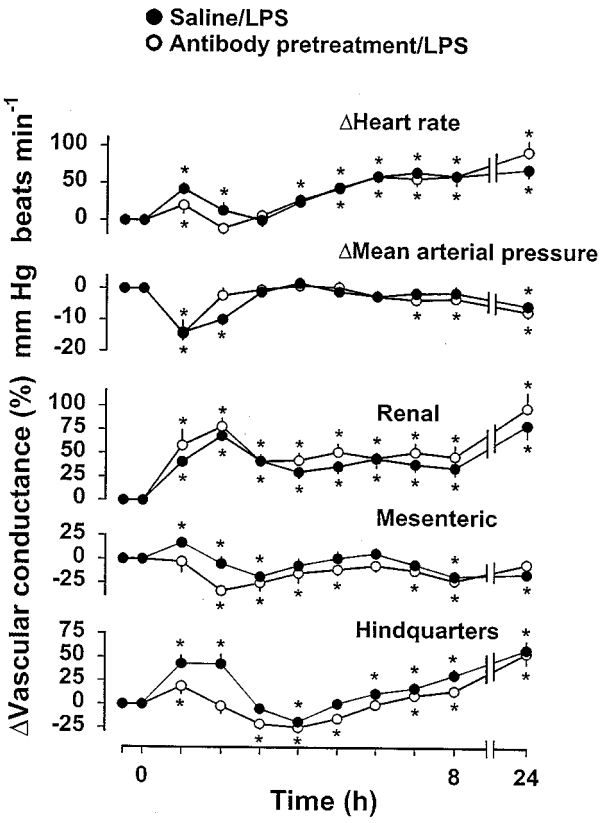

Figure 6 Cardiovascular changes in conscious Long Evans rats during a continuous infusion of lipopolysaccharide (LPS; 150 μg kg^{-1} h^{-1} starting at time 0), 1 h after pretreatment with saline ($n=9$) or a mixture of antibodies to TNF-α plus IL-1β (300 mg kg^{-1}; $n=8$). The vertical bars show s.e.mean; *$P<0.05$ versus original baseline (Friedman's test). Note the variation in the time scale.

1994, for review; Lin *et al*., 1997). As for TNF-α, the wide-ranging actions of IL-1β, involving simultaneous stimulation of pressor and depressor mechanisms (e.g., Gulick *et al*., 1989; Yoshizumi *et al*., 1990; Niijima *et al*., 1991; Robert *et al*., 1992; Morimoto *et al*., 1992; Bataillard & Sassard, 1994; Watanabe *et al*., 1996; see Dinarello, 1994, for review) likely account for the complexity of its haemodynamic effects, and our inability to detect a dose-dependency in the haemodynamic effects of IL-1β. However, it is surprising we saw only depressor responses to IL-1β over a dose range of 1–100 μg kg^{-1}, since this range covers the doses used by those who have reported only pressor effects of IL-1β (Morimoto *et al*., 1992; Bataillard & Sassard, 1994; Watanabe *et al*., 1996). In spite of this clear difference, we did observe biphasic increases in HR in response to IL-1β, as found by Morimoto *et al*. (1992), Bataillard & Sassard (1994), and Watanabe *et al*. (1996).

As mentioned above, IL-1β, like TNF-α, caused changes in regional vascular conductances indicative of activation of dilator and constrictor mechanisms. Such opposing actions may explain why Lin *et al*. (1997) failed to observe any significant effect of IL-1β on total peripheral resistance. In our experimental model, any lesser ability of IL-1β to cause arteriolar vasodilatation (Baudry *et al*., 1996) was not apparent, although greater reduction in noradrenaline sensitivity following IL-1β compared to TNF-α (Baudry *et al*., 1996) could have contributed to the overall similarity in the regional haemodynamic changes following the two cytokines. Since we used recombinant cytokines that were obtained from an *E. coli* expression system, we cannot exclude the possibility of low level contamination with LPS (see Rothwell *et al*., 1996). However, it is unlikely that LPS was responsible for the effects of TNF-α and IL-1β reported here since they differed in several respects from those seen following administration of LPS by bolus injection or by infusion.

Considering the relatively modest haemodynamic effects of TNF-α and IL-1β alone, their super-additive effect on co-administration (see Dinarello, 1994; Tracey & Cerami, 1994, for review) was clearly apparent. Interestingly, while the nadir of the initial fall in MAP was similar to the sum of the maximal hypotensive effects of TNF-α and IL-1β separately, the integrated hypotensive and associated renal vasodilator effects seen with co-administration of the cytokines were significantly greater than additive. Thereafter, there were substantial reductions in MVC and HVC, that were not seen with either cytokine alone, and these effects were accompanied by a rapid recovery in MAP to above resting levels. The profile and time course of these effects (see Gardiner *et al*., 1996b,c) are consistent with a possible synergistic interaction of TNF-α and IL-1β to enhance endothelin production (see Corder *et al*., 1995). The relatively greater reductions in MVC and HVC, and the less sustained increase in RVC, following co-administration of cytokines in the pretreatment, compared to the premixing schedules (compare Figures 4 and 5), was probably due to the greater dilution of the cytokines with saline in the premixing schedule.

By 24 h after co-administration of the cytokines there was an increase in HR, a decrease in MAP, and a hindquarters vasodilatation, while no such effects were apparent after TNF-α or IL-1β alone. In the light of the evidence that TNF-α and IL-1β activate the same set of transcription factors (see Eder, 1997, for review), such synergism is not unexpected, although the finding that cardiovascular consequences of interactions between TNF-α and IL-1β can occur 24 h after administration of the cytokines is notable. Previously we have reported that the marked renal and hindquarters vasodilatation, seen 24 h after the onset of LPS infusion, is not accompanied by increased activity of inducible nitric oxide synthase (Gardiner *et al*., 1995), and recently we provided evidence that the inducible form of heme oxygenase (HO-1) might be responsible for the delayed vasodilatation (Tomlinson *et al*., 1998). Hence, it would be of interest to determine if the synergism between TNF-α and IL-1β results in delayed expression of HO-1.

Co-administration of TNF-α and IL-1β produced initial changes in HR and MAP that were little different from those following bolus injection of LPS. However, the early nadir in MAP following LPS bolus was not accompanied by regional vasodilatation, so, presumably the hypotension was due to a fall in cardiac output, unlike the picture seen with LPS infusion, when cardiac output shows a marked increase (Gardiner *et al*., 1995). In experiments in which bolus administration of LPS is used as a model of endotoxaemia, it is generally assumed that any fall in MAP is a sign of vasodilatation (e.g., Szabó *et al*., 1993). The present findings indicate that such an assumption could be misleading, at least during the early period after bolus injection of LPS.

During the 1–8 h after bolus injection of LPS, the recovery in MAP was less brisk and to a slightly lower level than seen with the co-administration of TNF-α and IL-1β, and there was no accompanying fall in mesenteric vascular conductance after LPS, in contrast to the substantial fall seen after the cytokines. As alluded to above, one likely explanation for this difference is that co-administration of TNF-α and IL-1β causes more effective stimulation of endothelin production than does bolus

injection of LPS, at least under the conditions of our experiments.

By 24 h after bolus injection of LPS, the tachycardia and hindquarters vasodilatation were significantly less than after co-administration of the cytokines, but otherwise haemodynamics in the two groups were remarkably similar. Clearly any differences could be attributed to imperfect matching of the doses of LPS and cytokines, and in that sense we cannot dismiss the possibility that, in another protocol, it would be possible to simulate faithfully the full haemodynamic sequelae of bolus injection of LPS by co-administration of TNF-α and IL-1β.

Pretreatment with antibodies to TNF-α and IL-1β at a dose of 3 mg kg^{-1} had no discernible effects on the cardiovascular responses to the cytokines. Clearly, the antibodies were active, since at a dose of 300 mg kg^{-1} they prevented the hypotensive and mesenteric and hindquarters vasodilator effects of, and delayed the development of the renal vasodilator response to, co-administration of the cytokines. The relative resistance to antibody pretreatment of the renal vasodilator response to the cytokines may have been due, in part, to incomplete binding of the latter to the antibodies *in vivo*, since premixing cytokines and antibodies caused almost complete inhibition of the renal vasodilatation. However, even under these conditions, the antibodies did not prevent the delayed (at 24 h) tachycardia caused by the cytokines; the explanation for this is unclear, but there is some evidence that anti-cytokine antibodies can act as carrier proteins, thereby prolonging the *in vivo* effects of exogenous cytokines (Finkelman *et al*., 1993).

Against the background of this series of observations, the failure of antibody pretreatment to influence many of the cardiovascular consequences of LPS infusion is capable of several different interpretations. One is that the antibodies do not bind to rat cytokines, but this seems unlikely, because the same antibodies caused substantial inhibition of the cardiovascular changes, and elevation in plasma cytokine levels, in anaesthetized rats following bolus injection of LPS (Ruetten *et al*., 1996). In the light of this observation it could be suggested that bolus injection of LPS activates cytokine cascades in a way not seen with LPS infusion. However, we have shown that LPS infusion (as here) causes substantial elevation of plasma TNF-α levels (Waller *et al*., 1995). So, another interpretation is that only those effects of LPS infusion that were changed by antibody pretreatment (i.e., the initial hindquarters vasodilatation and the recovery from the initial fall in MAP) involved TNF-α and/or IL-1β. In a previous study, using hamster/murine chimeric anti-TNF monoclonal antibodies we observed little effect on the cardiovascular sequelae of LPS infusion, and suggested the majority of changes were due to factors other than plasma TNF (Waller *et al*., 1995). A similar conclusion with regard to the involvement of TNF-α in the hypotensive effect of bolus injection of LPS in anaesthetized rats has been reached recently by Xie *et al*. (1997). However, neither they, nor we, from our present or previous (Waller *et al*., 1995) results, can discount the possibility that the relative lack of effect of antibodies to cytokines in models of endotoxaemia are a reflection of the inability of the antibodies to gain access to local sites of synthesis and action of the endogenous cytokines. Indirect support for this proposal could be taken from the recent findings of Yamamoto *et al*. (1997) who reported that FR 167653, which inhibits the production of TNF-α and IL-1β *in vivo*, reduced the mortality and hypotension caused by LPS in rabbits.

Whatever the explanation of the relative lack of effect of cytokine antibodies on the cardiovascular sequelae of experimental endotoxaemia, the present results provide no evidence that they might be clinically useful in the treatment of septic shock.

References

BATAILLARD, A. & SASSARD, J. (1994). Cardiovascular effects of human recombinant interleukin-1β in conscious rats. *Am. J. Physiol.,* **266,** R1148 – R1153.

BAUDRY, N., RASETTI, C. & VICAUT, E. (1996). Differences between cytokine effects in the microcirculation of the rat. *Am. J. Physiol.,* **271,** H1186 – H1192.

BAUE, A.E. (1996). MOF/MODS, SIRS: an update. *Shock,* **6,** S1 – S5.

BAZZONI, F. & BEUTLER, B. (1996). The tumor necrosis factor ligand and receptor families. *New Eng. J. Med.,* **334,** 1717 – 1725.

BERNARD, G.R. (1995). Sepsis Trials: intersection of investigation, regulation, funding and practice. *Am. J. Respir. Crit. Care Med.,* **152,** 4 – 10.

BLACKWELL, T.S. & CHRISTMAN, J.W. (1996). Sepsis and cytokines: current status. *Br. J. Anaesth.,* **77,** 110 – 117.

CAVAILLON, J.-M., MUNOZ, C., FITTING, C., MISSET B. & CARLET, J. (1992). Circulating cytokines: the tip of the iceberg? *Circ. Shock,* **38,** 145 – 152.

CORDER, R., CARRIER, M., KHAN, N., KLEMM, P. & VANE, J.R. (1995). Cytokine regulation of endothelin-1 release from bovine aortic endothelial cells. *J. Cardiovasc. Pharmacol.,* **26** (Suppl. 3), S56 – S58.

DEITCH, E.A. (1998). Animal models of sepsis and shock: a review and lessons learned. *Shock,* **9,** 1 – 11.

DINARELLO, C.A. (1994). Interleukin-1. *Adv. Pharmacol.,* **25,** 21 – 51.

EDER, J. (1997). Tumour necrosis factor α and interleukin 1 signalling: do MAPKK kinases connect it all? *Trends Pharmacol. Sci.,* **18,** 319 – 322.

FINKELMAN, F.D., MADDEN, K.B., MORRIS, S.C., HOLMES, J.M., BOIANI, N., KATONA, I.M. & MALISZEWSKI, C.R. (1993). Anti-cytokine antibodies as carrier proteins: prolongation of *in vivo* effects of exogenous cytokines by injection of cytokine-anti-cytokine antibody complexes. *J. Immunol.,* **151,** 1235 – 1244.

FOULKES, R. & SHAW, S. (1992). The cardiodepressant and vasodepressant effects of tumour necrosis factor in rat isolated atrial and aortic tissues. *Br. J. Pharmacol.,* **106,** 942 – 947.

GALLEY, H.F. & WEBSTER, N.R. (1996). The immuno-inflammatory cascade. *Br. J. Anaesth.,* **77,** 11 – 16.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1995). Cardiac and regional haemodynamics, inducible nitric oxide synthase (NOS) activity, and the effects of NOS inhibitors in conscious, endotoxaemic rats. *Br. J. Pharmacol.,* **116,** 2005 – 2016.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996a). Effects of dexamethasone and SB 209670 on the regional haemodynamic responses to lipopolysaccharide in conscious rats. *Br. J. Pharmacol.,* **118,** 141 – 149.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996b). Influence of aminoguanidine and the endothelin antagonist SB 209670, on the regional haemodynamic effects of endotoxaemia in conscious rats. *Br. J. Pharmacol.,* **118,** 1822 – 1828.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1996c). Temporal differences between the involvement of angiotensin II and endothelin in the cardiovascular responses to endotoxaemia in conscious rats. *Br. J. Pharmacol.,* **119,** 1619 – 1627.

GARDINER, S.M., KEMP, P.A., MARCH, J.E., WOOLLEY, J. & BENNETT, T. (1998a). Effects of sheep polyclonal antibodies to human TNF-α and IL-1β on cardiovascular responses to the cytokines in conscious rats. *Br. J. Pharmacol.,* **123,** 308P.

GARDINER, S.M., KEMP, P.A., MARCH, J.E., WOOLLEY, J. & BENNETT, T. (1998b). Effect of sheep antibodies to human TNF-α and IL-1β on cardiovascular responses to lipopolysaccharide (LPS) in conscious rats. *Br. J. Pharmacol.,* **123,** 309P.

GARDINER, S.M., KEMP, P.A., WOOLLEY, J. & BENNETT, T. (1997a). Regional vasodilator responses to lipopolysaccharide compared to tumour necrosis factor-α plus interleukin-1β in conscious rats. *J. Vasc. Res.,* **34** (Suppl. 1), 17.

GARDINER, S.M., KEMP, P.A., WOOLLEY, J. & BENNETT, T. (1997b). Hypotensive and vasodilator responses to human recombinant interleukin-1β in conscious rats. *J. Vasc. Res.,* **34** (Suppl. 1), 18.

GOTO, K., HAMA, H. & KASUYA, Y. (1996). Molecular pharmacology and pathophysiological significance of endothelin. *Jpn. J. Pharmacol.,* **72,** 261 – 290.

GULICK, T., CHUNG, M.K., PIEPER, S.J., LANGE, L.G. & SCHREINER, G.F. (1989). Interleukin 1 and tumor necrosis factor inhibit cardiac myocyte β-adrenergic responsiveness. *Proc. Natl. Acad. Sci. U.S.A.,* **86,** 6753 – 6757.

LIN, M.T., LIU, H.H. & YANG, Y.L. (1997). Involvement of interleukin-1 receptor mechanisms in development of arterial hypotension in rat heatstroke. *Am. J. Physiol.,* **273,** H2072 – H2077.

MORIMOTO, K., MORIMOTO, A., NAKAMORI, T., TAN, N., MINAGAWA, T. & MURAKAMI, N. (1992). Cardiovascular responses induced in free-moving rats by immune cytokines. *J. Physiol.,* **448,** 307 – 320.

NEILSON, I.R., NEILSON, K.A., YUNIS, E.J. & ROWE, M.I. (1989). Failure of tumor necrosis factor to produce hypotensive shock in the absence of endotoxin. *Surgery,* **106,** 439 – 443.

NIIJIMA, A., HORI, T., AOU, S. & OOMURA, Y. (1991). The effects of interleukin-1β on the activity of adrenal, splenic and renal sympathetic nerves in the rat. *J. Auton. Nerv. Syst.,* **36,** 183 – 192.

PARILLO, J.E. (1993). Pathogenetic mechanisms of septic shock. *New Eng. J. Med.,* **328,** 1471 – 1477.

ROBERT, R., CHAPELAIN, B., JEAN, T. & NELIAT, G. (1992). Interleukin-1 impairs both vascular contraction and relaxation in rabbit isolated aorta. *Biochem. Biophys. Res. Commun.,* **182,** 733 – 739.

ROTHWELL, N.J., LUHESHI, G. & TOULMOND, S. (1996). Cytokines and their receptors in the central nervous system: physiology, pharmacology, and pathology. *Pharmacol. Ther.,* **69,** 85 – 95.

RUETTEN, H., SMITH, D., THIEMERMANN, C. & VANE, J.R. (1996). Effects of polyclonal antibodies against TNFα or IL-1β on the multiple organ failure syndrome elicited by endotoxin in the anaesthetised rat. *Br. J. Pharmacol.,* **119,** 19P.

SZABÓ, C., THIEMERMANN, C. & VANE, J.R. (1993). Inhibition of the production of nitric oxide and vasodilator prostaglandins attenuates the cardiovascular response to bacterial endotoxin in adrenalectomized rats. *Proc. Roy. Soc. Lond. B.,* **253,** 233 – 238.

TAKAHASHI, K., ANDO, K., ONO, A., SHIMOSAWA, T., OGATA, E. & FUJITA, T. (1992). Tumor necrosis factor-α induces vascular hyporesponsiveness in Sprague-Dawley rats. *Life Sci.,* **50,** 1437 – 1444.

THEODORSSON-NORHEIM, E. (1987). Friedman and Quade tests: BASIC computer program to perform non-parametric two-way analysis of variance and multiple comparisons on ranks of several related samples. *Comput. Biol. Med.,* **17,** 85 – 99.

TOMLINSON, A., GARDINER, S.M., WILLIS, D., ALI, J., KEMP, P.A., WILLOUGHBY, D.A. & BENNETT, T. (1998). Temporal and spatial expression of the inducible isoforms of cyclooxygenase, nitric oxide synthase and heme oxygenase in tissues from rats infused with LPS in the conscious state. *Br. J. Pharmacol.,* **123,** 178P.

TRACEY, K.J. & CERAMI, A. (1994). Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. *Annu. Rev. Med.,* **45,** 491 – 503.

TURNER, C.R., ESSER, K.M., WHEELDON, E.B., SLIVJAK, M. & SMITH, E.F. (1989). Cardiovascular and pulmonary effects of human recombinant tumor necrosis factor in the conscious rat. *Circ. Shock,* **28,** 369 – 384.

WALLER, J., GARDINER, S.M., JOSE, J. & BENNETT, T. (1995). Lack of effect of TNF antibodies on the cardiovascular sequelae of lipopolysaccharide infusion in conscious rats. *Br. J. Pharmacol.,* **116,** 2487 – 2495.

WANG, P., BA, Z.F. & CHAUDRY, I.H. (1994). Administration of tumor necrosis factor-α *in vivo* depresses endothelium-dependent relaxation. *Am. J. Physiol.,* **266,** H2535 – H2541.

WATANABE, T., TAN, N., SAIKI, Y., MAKISUMI, T. & NAKAMURA, S. (1996). Possible involvement of glucocorticoids in the modulation of interleukin-1-induced cardiovascular responses in rats. *J. Physiol.,* **491,** 231 – 239.

XIE, J., JOSEPH, K.O., BAGBY, G.J., GILES, T.D. & GREENBERG, S.S. (1997). Dissociation of TNF-α from endotoxin-induced nitric oxide and acute-phase hypotension. *Am. J. Physiol.,* **273,** H164 – H174.

YAMAMOTO, N., SAKAI, F., YAMAZAKI, H., SATO, N., NAKAHARA, K. & OKUHARA, M. (1997). FR167653, a dual inhibitor of interleukin-1 and tumor necrosis factor-α, ameliorates endotoxin-induced shock. *Eur. J. Pharmacol.,* **327,** 169 – 175.

YOSHIZUMI, M., KURIHARA, H., MORITA, T., YAMASHITA, T., OHHASHI, Y., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T. & YAZAKI, Y. (1990). Interleukin 1 increases the production of endothelin-1 by cultured endothelial cells. *Biochem. Biophys. Res. Commun.,* **166,** 324 – 329.

(Received April 30, 1998
Revised September 21, 1998
Accepted September 25, 1998)