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Cardiac and regional haemodynamics, inducible nitric oxide synthase (NOS) activity, and the effects of NOS inhibitors in conscious, endotoxaemic rats

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1 A reproducible model of the hyperdynamic circulatory sequelae of endotoxaemia in conscious, chronically-instrumented Long Evans rats, was achieved with a continuous infusion of lipopolysaccharide (LPS, 150 μg kg^{-1} h^{-1}) for 32 h. Over the first 2 h of LPS infusion, there was a transient hypotension and tachycardia, accompanied by a marked increase in renal flow and vascular conductance, although there were reductions in cardiac and stroke index. Between 4-8 h after the start of LPS infusion, there was slight hypotension and tachycardia, and a transient rise in mesenteric flow and conductance, but reductions in the hindquarters vascular bed; the hyperaemic vasodilatation in the renal vascular bed was maintained. At this stage, all cardiac haemodynamic variables and total peripheral conductance, were increased, but central venous pressure was reduced. By 24 h after the onset of LPS infusion, there was clear hypotension and tachycardia, accompanied by increases in renal and hindquarters flow and conductance, although mesenteric haemodynamic variables were not different from baseline. At this stage, cardiac and stroke index were substantially elevated, in association with marked increases in peak aortic flow, dF/dt_{max} and total peripheral conductance; these changes were well-maintained over the following 8 h of LPS infusion.

2 By 2 h after the start of LPS infusion, only lung inducible nitric oxide synthase (iNOS) activity was increased, but at 6 h there were significant increases in iNOS activity in lung, liver, spleen, heart and aorta (43.3 ± 7.8, 28.8 ± 3.3, 50.8 ± 7.2, 3.04 ± 0.29, 3.76 ± 0.94 pmol min^{-1} mg^{-1} protein, respectively). However, by 24 h after the start of LPS infusion, iNOS activity was not elevated significantly in any tissue examined, and kidney iNOS activity did not change significantly during LPS infusion. Plasma nitrite/nitrate levels were increased after 2 h infusion of LPS (from 6.07 ± 1.23 to 29.44 ± 7.08 μmol l^{-1}), and further by 6 h (228.10 ± 29.20 μmol l^{-1}), but were less 24 h after onset of LPS infusion (74.96 ± 11.34 μmol l^{-1}). Hence, the progressive hypotension, increasing cardiac function and developing hyperaemic vasodilatation in renal and hindquarters vascular beds between 8-24 h after the onset of LPS infusion, occurred when tissue iNOS activity and plasma nitrite/nitrate levels were falling.

3 Pretreatment with N^G-monomethyl-L-arginine (L-NMMA, 30 mg kg^{-1} bolus, 30 mg kg^{-1} h^{-1} infusion) 1 h before LPS infusion did not prevent the early hypotension, but abolished the initial renal vasodilatation and the later (6-8 h) fall in mean arterial pressure (MAP), and the additional renal vasodilatation. However, under these conditions, mesenteric and hindquarters flows and conductances were substantially decreased. Similar, but less marked, effects were seen with L-NMMA pretreatment at 10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion, whereas at a lower dose of 3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion, L-NMMA pretreatment had little effect on responses to LPS.

4 Delaying treatment with L-NMMA (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) until 4 h after the start of LPS infusion prevented the late hindquarters vasodilatation and attenuated the late renal vasodilatation, but still reduced mesenteric flow. When treatment with L-NMMA was delayed until 24 h after the start of LPS infusion, renal and hindquarters vasodilatations were only slightly affected, but mesenteric flow was still compromised. Delayed treatment with L-NAME (3 mg kg^{-1} h^{-1} starting 24 h after onset of LPS infusion) caused substantial inhibition of the renal vasodilatation, but also caused marked reduction in mesenteric and hindquarters flows and indices of cardiac performance.

5 These findings indicate that iNOS activity is not directly responsible for the widespread vasodilatation seen after 24 h infusion of LPS in conscious rats. If our observations can be extrapolated to the clinical situation, they indicate that non-selective NOS inhibition could have detrimental effects in endotoxaemic patients with signs of a hyperdynamic circulation.

Keywords: Lipopolysaccharide; regional haemodynamics; cardiac function; inducible nitric oxide synthase activity; NOS inhibitors

Introduction

The administration of bacterial lipopolysaccharide (LPS) to animals (for review see Redl *et al.*, 1993) or man (Suffredini *et al.*, 1989; for review, see Parrillo, 1993) has been employed as a means of simulating the systemic response to infection. However, the marked inter-species differences in the susceptibility to LPS (Redl *et al.*, 1993) means that there is no ideal model of the clinical condition. But the substantial inter-individual variation in the development of the systemic inflammatory response syndrome (SIRS) or sepsis (Bone, 1993) in man (Guillou, 1993) means that there is no single clinical entity to be modelled. It follows, therefore, that the most useful experimental approach would simulate at least some of the characteristics of the clinical condition, and subsequently allow dissection of the contributory factors. Although administra-

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tion of LPS causes dysfunction in all organ systems examined (Parrillo, 1993) our particular interest is in the cardiovascular sequelae of LPS administration.

In the clinical setting, SIRS, sepsis, or the multiple organ dysfunction syndrome (MODS) (Bone, 1993) are relatively slow in onset, whereas, in the majority of studies in rats, a large dose of LPS has been given which evokes a rapid-onset hypotension (e.g. Brackett *et al.*, 1985; Schaller *et al.*, 1985; Guc *et al.*, 1990; Thiemermann & Vane, 1990; Szabó *et al.*, 1993; Paya *et al.*, 1993). It is known that infusion of LPS at lower doses does not have an immediate hypotensive effect (Fish *et al.*, 1986; Julou-Schaeffer *et al.*, 1990), and so we considered it possible that chronic infusion of a low dose of LPS in conscious, unrestrained rats might better mimic the clinical situation, and also allow quantitation of the chronological profile of changes in regional and cardiac haemodynamics. This approach has been employed successfully in recent studies on the effects of LPS on cardiac haemodynamics in conscious sheep (Traber *et al.*, 1988; Sugi *et al.*, 1991; Meyer *et al.*, 1992; Weber *et al.*, 1992; Noshima *et al.*, 1993).

While it is clear that the responses to LPS administration involve a multiplicity of factors, particular interest has focused on the possibility that inhibition of nitric oxide (NO) production might exert beneficial effects in this circumstance (Rees *et al.*, 1990; Thiemermann & Vane, 1990; Kilbourn *et al.*, 1990; Julou-Schaeffer *et al.*, 1990; Gray *et al.*, 1991; Wright *et al.*, 1992; Meyer *et al.*, 1992; Schott *et al.*, 1993; Paya *et al.*, 1993; Mitchell *et al.*, 1993), or in the clinical treatment of SIRS, sepsis or MODS (Petros *et al.*, 1991; 1994; Schneider *et al.*, 1992; Lorente *et al.*, 1993). However, intervention with non-selective NO synthase (NOS) inhibitors can have detrimental effects following administration of LPS (Wright *et al.*, 1992; Cobb *et al.*, 1992; 1995). Against this background, the present work had four objectives:- (1) to delineate the changes in regional and cardiac haemodynamics during infusion of LPS, or saline, over a period of 32 h in conscious rats; (2) to measure tissue inducible NOS (iNOS) activity and plasma nitrite/nitrate levels at selected time-points during LPS infusion; (3) to assess the effects on regional haemodynamics of administering different doses of the non-selective NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA) before, or 4, or 24 h after the onset of LPS infusion, and (4) to determine if treatment with the non-selective NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), 24 h after the start of LPS infusion, had effects different from those of L-NMMA. Some of the work described here has been communicated to the British Pharmacological Society (Gardiner *et al.*, 1992; 1994a,b; Bennett *et al.*, 1995).

Methods

All experiments were carried out on male, Long Evans rats (350–450 g) bred in the Biomedical Services Unit in Nottingham. All surgery was carried out under anaesthesia (sodium methohexitone (Brietal, Lilly) 40–60 mg kg^{-1}, i.p., supplemented as required). The procedures for implanting pulsed Doppler probes, or electromagnetic flow probes, and intravascular catheters have been described in detail previously (Gardiner *et al.*, 1993; 1994c; Waller *et al.*, 1994a). Briefly, probes were implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta (to monitor flow through the hindquarters). Catheters were implanted in the right jugular vein (for i.v. infusions) and in the distal abdominal aorta, via the ventral caudal artery (for measurement of intra-arterial pressure and heart rate). Flow probes were always implanted at least 7 days before catheterization, and the experiments began at least 24 h later.

Regional haemodynamic studies

Thirteen different groups of rats were studied as follows:-

Group A ($n=8$): were given a continuous i.v. infusion of sterile isotonic saline (154 mmol l^{-1} NaCl) for 32 h. Baseline recordings were made for 30 min before the start of the infusion (06 h 30 min–07 h 00 min) and for 10 min across each 1 h time point for the subsequent 8 h (i.e. until 15 h 00 min). Recordings were begun again at 07 h 00 min the next day (i.e. 24 h after the start of saline infusion) and were made across each 1 h time point, as above, for the next 8 h. These recording time points were kept constant throughout all protocols, although the durations of the experiments varied, as described below.

Group B ($n=8$): were given a continuous infusion of LPS (15 μg kg^{-1} h^{-1} in 0.4 ml h^{-1} saline) for 24 h. This low dose of LPS had somewhat variable effects (see Results), so in all subsequent protocols a 10 fold higher dose of LPS was used.

Group C ($n=8$): were given a continuous infusion of LPS (150 μg kg^{-1} h^{-1}) for 24 h, and LPS together with saline (0.4 ml h^{-1}) for the subsequent 8 h. The additional infusion of saline was to control for L-NAME infusion in Group M.

Group D ($n=8$): were given a continuous infusion of saline (to control for L-NMMA infusion in Groups E-I) beginning 1 h before onset of LPS infusion for 23 h.

Group E ($n=4$): were given L-NMMA (30 mg kg^{-1} bolus, 30 mg kg^{-1} h^{-1} infusion) beginning 1 h before onset of saline infusion (to control for LPS in Groups D and F) for 7 h.

Group F ($n=5$): were given L-NMMA (30 mg kg^{-1}, 30 mg kg^{-1} h^{-1}) beginning 1 h before onset of LPS for 7 h.

Group G ($n=7$): were given L-NMMA (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) beginning 1 h before onset of LPS for 7 h.

Group H ($n=8$): were given L-NMMA (3 mg kg^{-1}, 3 mg kg^{-1} h^{-1}) beginning 1 h before onset of infusion of saline for 23 h.

Group I ($n=8$): were given L-NMMA (3 mg kg^{-1}, 3 mg kg^{-1} h^{-1}) beginning 1 h before the start of infusion of LPS for 23 h.

Group J ($n=9$): were given an infusion of LPS for 24 h together with L-NMMA (10 mg kg^{-1}, 10 mg kg^{-1} h^{-1}) beginning 4 h after the onset of LPS infusion.

Group K ($n=8$): were given a saline infusion for 32 h together with L-NMMA (10 mg kg^{-1}, 10 mg kg^{-1} h^{-1}) for the last 8 h.

Group L ($n=8$): were given an infusion of LPS for 32 h, together with L-NMMA (10 mg kg^{-1}, 10 mg kg^{-1} h^{-1}) for the last 8 h.

Group M ($n=8$): were given an infusion of LPS for 32 h together with L-NAME (3 mg kg^{-1} h^{-1}) for the last 8 h.

Cardiac haemodynamic studies

Three different groups of rats were studied as follows:-

Group A ($n=8$): were given a continuous infusion of saline for 32 h.

Group B ($n=8$): were given a continuous infusion of LPS for 32 h together with saline for the last 8 h (to control for L-NAME in Group C).

Group C ($n=8$): were given a continuous infusion of LPS for 32 h, together with L-NAME (3 mg kg^{-1} h^{-1}) for the last 8 h.

Measurement of NOS activity and plasma nitrite/nitrate

Rats with i.v. catheters (as above) were given either a con-

tinuous infusion of sterile isotonic saline containing 15 units ml^{-1} heparin for 24 h, or an infusion of LPS for 2, 6 or 24 h. Blood samples (2 ml) were withdrawn from the arterial catheter and animals were then anaesthetized with halothane (5% in oxygen), decapitated and tissues removed. Tissues were frozen in liquid nitrogen, and stored together with the plasma samples at –80°C. Measurements of tissue NOS activity and plasma nitrite/nitrate levels were made at Wellcome Research Laboratories, according to the procedures described in detail by Rees *et al.* (1995).

Data analysis

Within-group analysis of haemodynamic data was by Friedman's test; the biochemical data were analysed by analysis of variance. A P value <0.05 was taken as significant. In the results, time values given in parentheses indicate points at which haemodynamic variables were significantly different from baseline levels.

Drugs

L-NMMA hydrochloride was a gift from Wellcome Research Laboratories; L-NAME hydrochloride and LPS (*E.coli* serotype 0127:B8) were purchased from Sigma (U.K.).

Results

The model of endotoxaemia

Regional haemodynamics (Groups A-C) Resting cardiovascular variables in the animals in this phase of the study are shown in Table 1; there were no significant differences between the groups. Throughout the 32 h of saline infusion, there were no significant changes in mean arterial blood pressure (MAP), heart rate or renal haemodynamics (Figure 1). However, there were significant reductions in mesenteric flow and conductance (2–8 h), but by 24 h mesenteric haemodynamic variables had returned to the pre-infusion baseline. Thereafter, reductions in mesenteric flow and conductance again occurred (26–32 h). The most likely explanation of this circadian variation in mesenteric haemodynamics is that monitoring began at the start of the light cycle, when the animals had a post-prandial mesenteric hyperaemia (being nocturnal feeders). This proposal is consistent with the finding that the circadian variation in hindquarters haemodynamics was less marked (Figure 1; see also Gardiner *et al.*, 1994c).

In the animals receiving LPS at 15 μg kg^{-1} h^{-1} there was a slight rise in MAP (at 3 h) and a biphasic tachycardia (at 2, 5–7 and 24 h) (Figure 1). There were marked and sustained increases in renal flow (1–24 h) and vascular conductance (2–24 h), whereas there were transient reductions in mesenteric flow and vascular conductance (both at 2 and 3 h), and hindquarters flow and vascular conductance (both at 4 h) (Figure 1).

Since LPS at 15 μg kg^{-1} h^{-1} did not cause hypotension at any time, all subsequent studies used LPS at a dose of 150 μg kg^{-1} h^{-1}, because after 24 h this dose produced a fall in MAP, albeit modest (see below).

During infusion of LPS at 150 μg kg^{-1} h^{-1} there was a biphasic fall in MAP (at 2 and 5–32 h) and tachycardia (at 1 and 4–32 h). These changes were accompanied by marked increases in renal flow and vascular conductance (2–32 h). Mesenteric flow and vascular conductance fell (both at 2 and 3 h) and then showed a slight increase (both at 6 h); thereafter the changes were not different from those in saline-infused animals (Figure 1). Hindquarters flow and vascular conductance showed some reduction (both at 3–5 h), but then a clear increase (both at 24–32 h) (Figure 1). Between 24 and 32 h, the hypotensive, tachycardic, and renal and hindquarters hyperaemic vasodilator effects of LPS were well maintained (Figure 1).

Table 1 Pretreatment resting cardiovascular variables in the 13 groups of rats instrumented for monitoring regional haemodynamics

	A ($n=8$)	B ($n=8$)	C ($n=8$)	D ($n=8$)	E ($n=4$)	F ($n=5$)	G ($n=7$)	H ($n=8$)	I ($n=8$)	J ($n=9$)	K ($n=8$)	L ($n=8$)	M ($n=8$)
Heart rate (beats min^{-1})	348±10	327±8	326±4	357±9	353±6	326±4	329±9	351±5	340±6	345±6	340±8	334±12	339±3
Mean blood pressure (mmHg)	103±2	101±2	100±2	105±2	106±1	107±2	105±2	103±2	104±1	103±1	105±2	102±2	103±2
Renal Doppler shift (kHz)	7.3±6	7.1±1.1	7.0±0.8	7.1±0.5	8.4±0.9	8.0±1.0	7.2±1.1	7.0±1.2	6.6±0.8	6.2±0.3	7.1±0.8	6.9±0.4	5.5±0.5
Mesenteric Doppler shift (kHz)	8.9±0.9	6.2±0.6	7.9±0.5	6.1±0.4	6.3±1.1	7.1±0.3	6.3±0.3	7.3±0.5	7.3±0.5	6.5±0.6	7.0±0.6	7.8±0.6	6.7±0.5
Hindquarters Doppler shift (kHz)	4.3±0.2	4.5±0.5	4.6±0.3	4.6±0.3	5.0±0.7	5.2±0.3	4.8±0.3	4.8±0.3	4.8±0.3	4.8±0.3	4.2±0.3	4.8±0.5	5.2±0.4
Renal vascular conductance ([kHz $mmHg^{-1}$]10^3)	71±5	69±9	70±7	68±5	79±9	75±10	68±9	67±11	63±8	60±3	67±6	68±4	53±4
Mesenteric vascular conductance ([kHz $mmHg^{-1}$]10^3)	87±9	62±7	80±6	59±4	59±10	67±4	59±2	71±5	70±6	63±6	67±6	77±7	65±5
Hindquarters vascular conductance ([kHz $mmHg^{-1}$]10^3)	42±3	45±5	46±3	44±3	47±6	49±3	46±3	47±3	46±3	46±3	40±3	47±5	51±5

Values are mean±s.e.mean; description of the groups is given in Methods.

Cardiac haemodynamics (Groups A and B) Resting cardiovascular variables are shown in Table 2; there were no significant differences between the groups.

During infusion of saline for 32 h there was a slight bradycardia (2–8 h) and a fall in dF/dt_{max} (at 3 h) but no other changes (Figure 2).

During infusion of LPS, there was a biphasic fall in MAP (at 1 and 2 h, and 6–32 h) and tachycardia (at 1 and 2 h and 5–32 h) (Figure 2), consistent with the changes seen in the animals instrumented for recording regional haemodynamics. There was a slight reduction in cardiac index (at 1 and 3 h), but thereafter a marked increase (4–32 h); stroke index showed a similar pattern of change (down at 1–3 h, and up at 5–32 h), as did peak aortic flow (down at 3 h, up at 5–32 h) (Figure 2). dF/dt_{max} showed an initial rise (at 2 h), but then fell (at 3 h) before showing a sustained increase (5–32 h). Total peripheral conductance increased (4–32 h), whereas central venous pressure was reduced initially (2–8 h), but subsequently was not different from baseline (Figure 2).

Tissue NOS activity There were no significant changes in constitutive (i.e., Ca^{2+}-dependent) NOS activity in any tissues taken from animals infused with LPS (data not shown). In contrast, there were clear increases in inducible (i.e., Ca^{2+}-independent) NOS (iNOS) activity in most tissues, although the patterns of change varied (Figure 3). It is notable that between 6 and 24 h after the start of LPS infusion, tissue iNOS activity fell, whereas over this time course the regional and

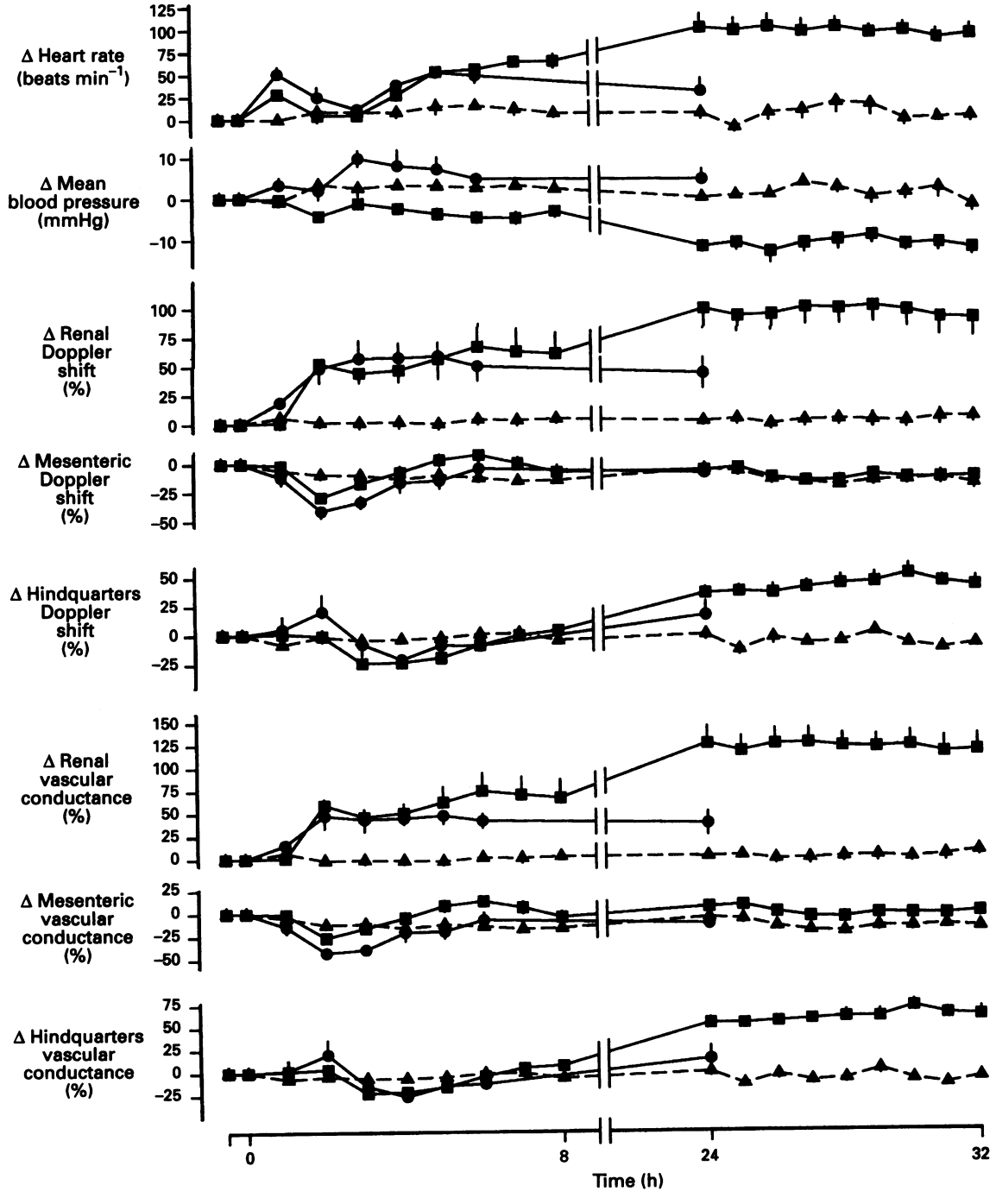

Figure 1 Regional haemodynamic changes during infusion of saline for 32 h (▲; $n=8$, Group A), or LPS (15 μg kg^{-1} h^{-1}, ●; $n=8$, Group B) for 24 h, or LPS (150 μg kg^{-1} h^{-1}) for 32 h together with saline from 24–32 h (■; $n=8$, Group C) in conscious Long Evans rats. Values are mean with s.e.mean; statistics are given in the text for clarity.

Table 2 Pretreatment, resting cardiovascular variables in the 3 groups of rats instrumented for monitoring cardiac haemodynamics

	A ($n=8$)	B ($n=8$)	C ($n=8$)
Heart rate (beats min^{-1})	381±11	365±6	380±8
Mean blood pressure (mmHg)	98±2	104±2	102±1
Cardiac index (ml min^{-1} 100 g^{-1})	26.1±1.4	23.7±1.0	26.5±0.6
Stroke index (μl $beat^{-1}$ 100 g^{-1})	69±3	65±2	70±2
Peak aortic flow (ml min^{-1} 100 g^{-1})	103±5	97±3	108±3
dF/dt_{max} (1 min^{-2} 100 g^{-1})	445±21	418±17	457±17
Total peripheral conductance (μl min^{-1} $mmHg^{-1}$ 100 g^{-1})	270±16	232±14	262±7
Central venous pressure (cmH_2O)	3.0±0.4	4.0±0.4	3.7±0.6

Values are mean±s.e.mean; description of the groups is given in Methods.

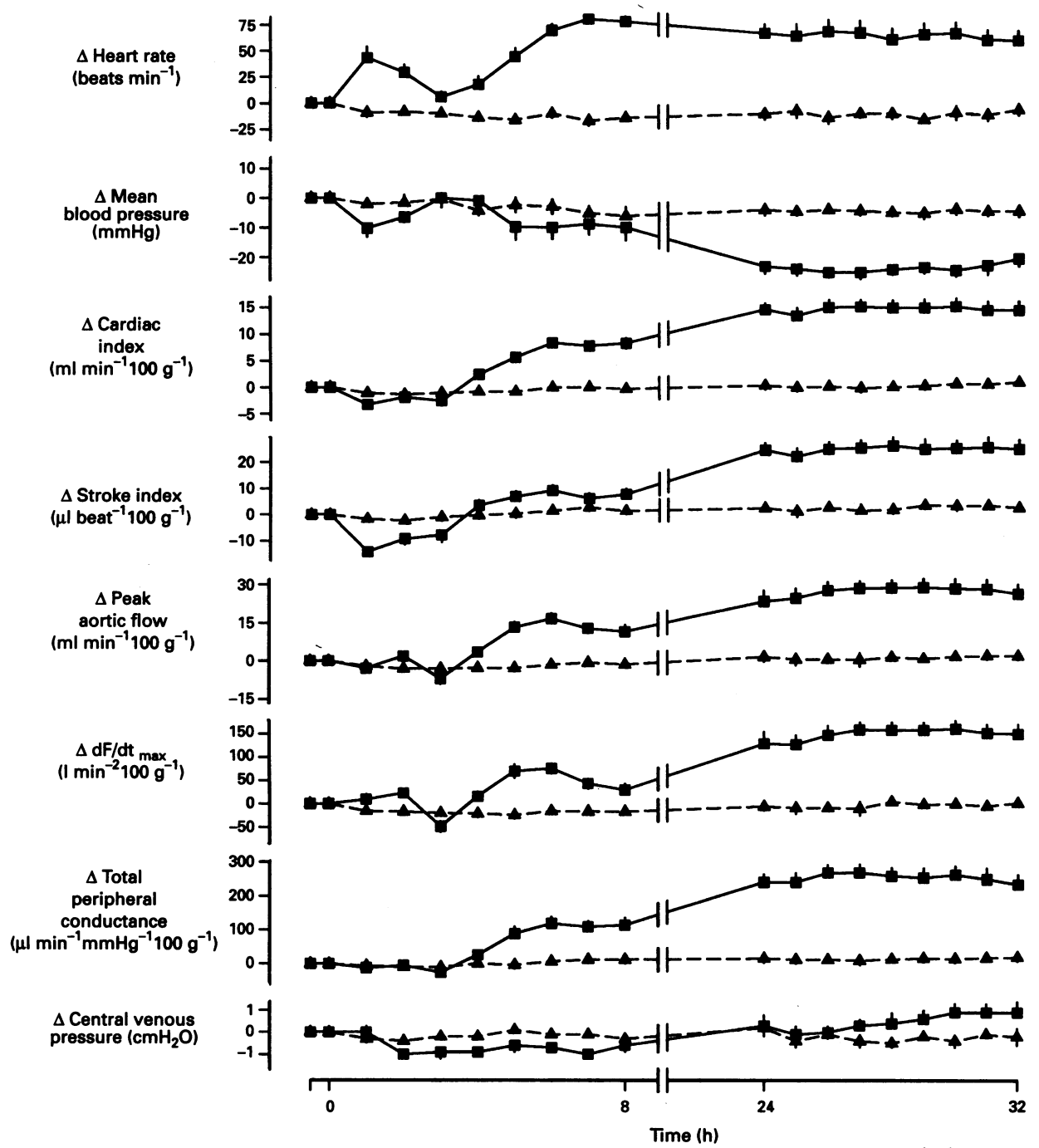

Figure 2 Cardiac haemodynamic changes during infusion of saline (▲; $n=8$, Group A) for 32 h, or LPS (150 μg kg^{-1} h^{-1}, ■; $n=8$, Group B) for 32 h together with saline for the last 8 h in conscious Long Evans rats. Values are mean with s.e.mean; statistics are given in the text for clarity.

cardiac haemodynamic sequelae of LPS infusion increased (Figures 1 and 2).

Plasma nitrite and nitrate (NO_x) During infusion of saline there were no significant changes in plasma NO_x levels (Table 3). However, during infusion of LPS there was a marked increase, although by 24 h after the onset of LPS infusion plasma NO_x levels were not as high as after 6 h (Table 3).

Effect of pretreatment with L-NMMA on regional haemodynamic responses to LPS

Effects of L-NMMA at 30 mg kg^{-1} bolus, 30 mg kg^{-1} h^{-1} infusion (Groups D-F) Resting cardiovascular variables of the animals in this protocol are shown in Table 1; there were no significant differences.

Since animals receiving L-NMMA at this dose together with LPS showed marked impairment of regional perfusion (see below), these protocols were run only for 8 h. In animals receiving L-NMMA and saline there was a marked increase in MAP and a bradycardia (both 1–8 h) (Figure 4). Although there was no change in renal flow, renal vascular conductance was decreased (1–8 h). In mesenteric and hindquarters vascular beds, flows and conductances were decreased (all 1–8 h) (Figure 4).

In animals receiving saline and LPS, changes were similar to those described above (Figure 1) i.e., a biphasic fall in MAP (at 1 and 2 h, and 5–7 h) and tachycardia (at 1 and 5–7 h), increases in renal flow (2–7 h) and vascular conductance (1–7 h), a decrease (at 3 and 4 h) followed by an increase (at 6 and 7 h) in mesenteric flow and vascular conductance, and a decrease in hindquarters flow and vascular conductance (both 3–5 h) (Figure 4).

In animals receiving L-NMMA prior to LPS, the effects of the former over the first 1 h were as described above (Figure 4). In the presence of L-NMMA, LPS caused a marked fall in MAP (back to baseline levels at 1 h), but this effect reversed within 2 h, and subsequently MAP remained at pre-LPS levels (3–7 h). There were no significant changes in renal flow, but renal vascular conductance was reduced (3–7 h) (Figure 4). In the presence of L-NMMA, LPS caused further reductions in mesenteric flow and vascular conductance (both 2–7 h) and hindquarters flow and vascular conductance (both 3–7 h) (Figure 4).

Effects of L-NMMA at 10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion (Group G) Resting cardiovascular variables for this

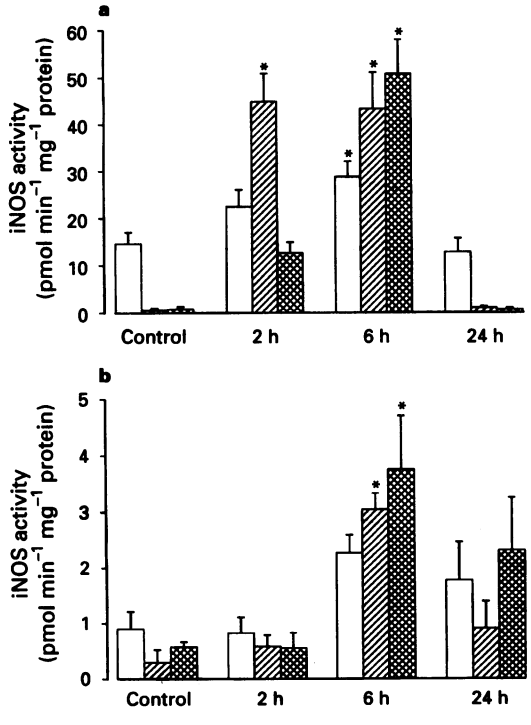

Figure 3 Tissue iNOS activity under control conditions (i.e., 24 h after start of saline infusion) and at the times indicated after onset of LPS infusion. (a) Open columns, liver; hatched columns, lung; cross-hatched columns, spleen. (b) Open columns, kidney; hatched columns, heart; cross-hatched columns, aorta. Values are mean with s.e.mean; $n=6-8$ animals in each group. *$P<0.05$ versus control (ANOVA followed by Duncan's test).

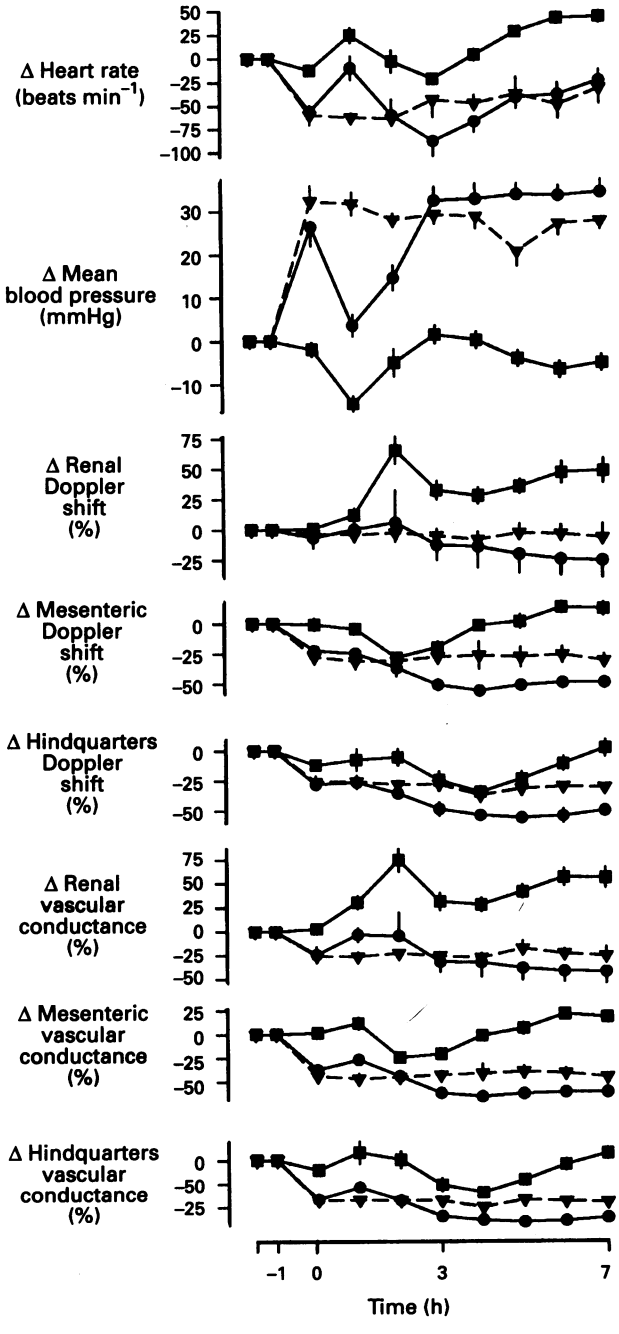

Figure 4 Regional haemodynamic changes in conscious, Long Evans rats receiving a continuous infusion of saline beginning 1 h before start of LPS infusion (150 μg kg^{-1} h^{-1} at time = 0h, ■, $n=8$; Group D) or N^G-monomethyl-L-arginine L-NMMA, 30 mg kg^{-1} bolus, 30 mg kg^{-1} h^{-1}) beginning 1 h before start of saline infusion (at time = 0h, ▼, $n=4$, Group E), or L-NMMA beginning 1 h before onset of LPS infusion (at time = 0h, ●, $n=5$, Group F). Values are mean with s.e.mean; statistics are given in the text for clarity.

group are shown in Table 1. One h after the start of administering L-NMMA at this dose there was a clear rise in MAP (19±3 mmHg) and a bradycardia (–21±6 beats min^{-1}) there was no change in renal flow (–6±4%), but a slight fall in renal vascular conductance (–10±4%). However, there were reductions in mesenteric and hindquarters flows (–14±3 and –22±6%, respectively) and vascular conductances (–27±3 and –34±5%, respectively). Under these conditions, LPS caused a transient fall in MAP (–17±3 mmHg at 1 h), but subsequently the pressor effect of L-NMMA was re-established (18±4 mmHg at 3 h), although there was no significant bradycardia. There was a transient rise in renal flow and vascular conductance (46±17 and 38±16%, respectively at 2 h), but subsequently a tendency towards a reduction in both variables. The L-NMMA-induced reductions in mesenteric and hindquarters flows and vascular conductances were augmented in the presence of LPS (mesenteric flow –33±5% and conductance –42±5% at 3 h; hindquarters flow –39±8% and conductance –42±9% at 3 h). Although these effects showed some diminution with time, the protocol was not extended beyond 8 h because the animals showed signs of malaise.

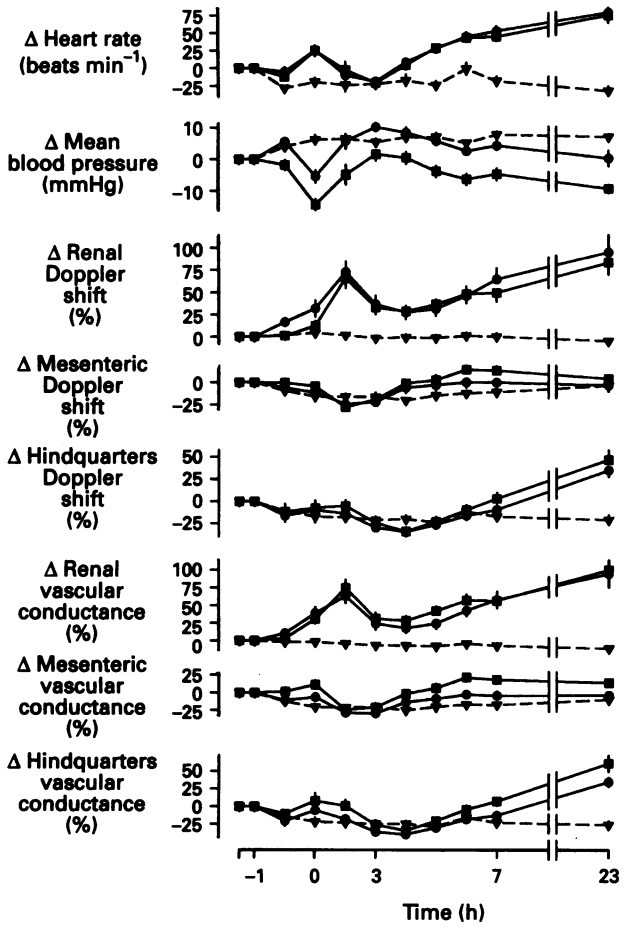

Figure 5 Regional haemodynamic changes in conscious Long Evans rats receiving a continuous infusion of saline beginning 1 h before start of LPS infusion (150 μg kg^{-1} h^{-1} at time=0h, ■, n=8; Group D) or N^G-monomethyl-L-arginine (L-NMMA, 3 mg kg^{-1} bolus, 3 mg kg^{-1} h^{-1} infusion) beginning 1 h before onset of saline infusion (at time=0h, ▼, n=8, Group H), or L-NMMA beginning 1 h before onset of LPS infusion (at time=0h, ●, n=8, Group I). Values are mean with s.e.mean: statistics are given in the text for clarity.

Effects of L-NMMA at 3 mg kg^{-1} bolus 3 mg kg^{-1} h^{-1} infusion (Groups H and I) Resting cardiovascular variables for these groups are shown in Table 1; there were no significant differences.

Administration of this dose of L-NMMA had a slight hypertensive and bradycardic action (both 0–23 h) (Figure 5). There were no significant changes in renal haemodynamics, but there were reductions in mesenteric and hindquarters flows and vascular conductances (all 0–23 h) (Figure 5). In the presence of L-NMMA, the initial hypotensive effect of LPS (at 1 h) was still present, but at 23 h MAP was not reduced below baseline, although it was lower than in the presence of LNMMA and saline (Figure 5). The tachycardia (at 1 h and 6–23 h) and renal hyperaemic vasodilatation (1–23 h) caused by LPS were unchanged in the presence of L-NMMA, as was the initial reduction 3–5 h) and subsequent increase (at 23 h) in hindquarters flow and vascular conductance (Figure 5).

Effects of delayed treatment with L-NMMA on regional haemodynamic responses to LPS

Effects of L-NMMA (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) beginning 4 h after the onset of LPS infusion (Group J) Resting cardiovascular variables are shown in Table 1.

Considering the relatively slight effects of the lower dose L-NMMA pretreatment (see above), and the profile of change in iNOS activity (Figure 3), it seemed feasible that delayed

Table 3 Plasma NO_x levels (μmol l^{-1}) during infusion of saline (n=5) or LPS (n=8)

	Saline	*LPS*
Control	6.89±2.59	6.07±1.23
2 h	7.51±1.63	29.44±7.08*
6 h	6.34±2.00	228.10±29.20*
24 h	5.06±2.14	74.96±11.34*

Values are mean±s.e.mean.
*P<0.05 versus control (ANOVA).

Table 4 Cardiovascular changes (relative to baseline) 4 h after the start of LPS infusion, and 1, 4 and 20 h after co-administration of LPS and N^G-monomethyl-L-arginine (L-NMMA 10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion)

	LPS	*LPS+L-NMMA*		
	4 h	5 h	8 h	24 h
Δ Heart rate (beats min^{-1})	–4±8	–12±7†	28±11†	69±16*†
Δ Mean blood pressure (mmHg)	4±1	13±2*†	14±1*†	8±1*†
Δ Renal Doppler shift (%)	26±7*	1±4†	5±9†	56±11*†
Δ Mesenteric Doppler shift (%)	14±5*	–18±4*†	–13±4*†	–22±4*†
Δ Hindquarters Doppler shift (%)	–40±3*	–39±4*	–17±5*†	6±9†
Δ Renal vascular conductance (%)	22±7*	–10±4†	–7±8†	47±10*
Δ Mesenteric vascular conductance (%)	10±5	–27±3*†	–23±3*†	–28±3*†
Δ Hindquarters vascular conductance (%)	–42±3*	–46±4*	–26±5*†	–1±9†

Values are mean±s.e.mean.
*P<0.05 versus baseline; † versus 4 h value (Friedman's test)

treatment with L-NMMA at 10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion might have beneficial effects. The results are shown in Table 4, and indicate that this regime of treatment with L-NMMA still augmented the mesenteric vasoconstrictor response to LPS, attenuated the late renal vasodilatation, and prevented the hindquarters vasodilatation.

Effects of L-NNMA (10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion) beginning 24 h after the onset of LPS infusion (Groups K and L) Resting cardiovascular variables are shown in Table 1; there were no significant differences.

As before, L-NMMA caused an increase in MAP and bradycardia (1–8 h, i.e. 25–32 h after the onset of saline infusion) (Figure 6). There were no changes in renal haemodynamics, but clear reductions in mesenteric and hindquarters flows and vascular conductances (all 1–8 h) (Figure 6).

Twenty four h after the start of LPS infusion, the haemodynamic status was as described above, i.e. there was a slight hypotension, clear tachycardia and increases in renal and hindquarters flow and conductance, but no change in mesenteric haemodynamics (Figure 6). Under these conditions, L-NMMA raised MAP (1–8 h) but not above baseline; there was a reduction in heart rate (at 1, 2, 4, 5, 7 and 8 h), but it was still above baseline. Renal flow and vascular conductance fell in response to L-NMMA (both 2–8 h), but were still substantially elevated (Figure 6). L-NMMA also diminished hindquarters flow (6–8 h) and vascular conductance (4–8 h), but did not abolish the effects of LPS (Figure 6). In the mesenteric vascular bed, in the presence of LPS, L-NMMA caused reductions in flow and conductance (both 1–8 h) (Figure 6).

In the presence of LPS, L-NMMA caused a smaller rise in MAP, and lesser reductions in mesenteric vascular conductance, and hindquarters flow and vascular conductance, than in the presence of saline (Figure 6). However, in the latter condition, L-NMMA caused significantly smaller reductions in renal flow and vascular conductance than in the presence of LPS (Figure 6).

Effects of delayed treatment with L-NAME (3 mg kg^{-1} h^{-1}) on regional and cardiac haemodynamic responses to LPS

We assessed regional and cardiac haemodynamic effects of L-NAME infusion, beginning 24 h after start of LPS infusion, to allow comparison with the effects of L-NMMA.

Regional haemodynamics (Group M) Resting cardiovascular variables are shown in Table 1.

L-NAME reversed LPS-induced hypotension (1–5 h) and produced a rise in MAP above baseline (6–8 h) (Figure 7).

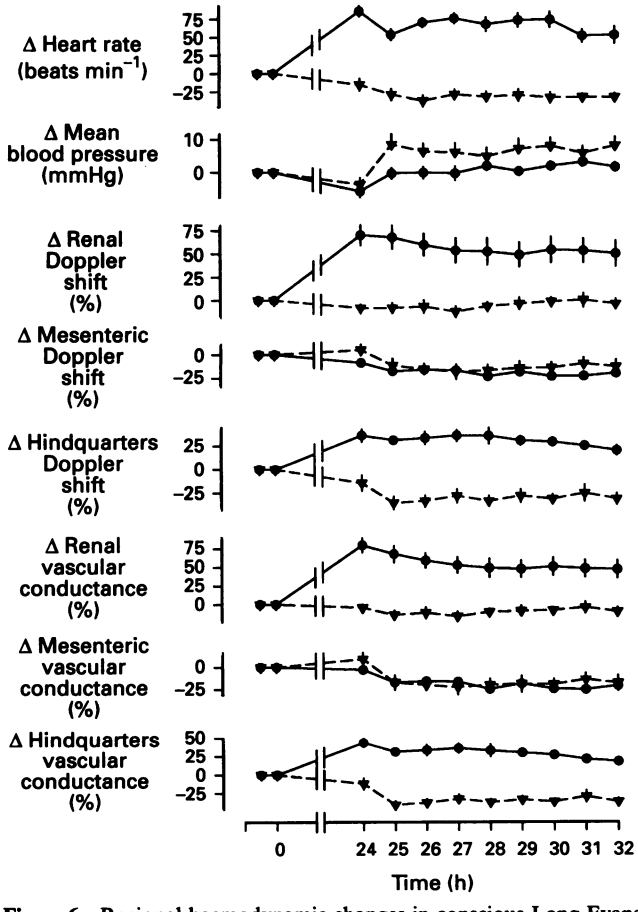

Figure 6 Regional haemodynamic changes in conscious Long Evans rats receiving saline infusion (0–32 h) together with N^G-monomethyl-L-arginine (L-NMMA, 10 mg kg^{-1} bolus, 10 mg kg^{-1} h^{-1} infusion from 24–32 h) (▼, $n=8$, Group K), or LPS infusion (150 μg kg^{-1} h^{-1}, 0–32 h) together with L-NMMA (from 24–32 h) (●, $n=8$, Group L). Values are mean with s.e.mean. Within-group statistics are given in the text for clarity; #$P<0.05$ for differences between integrated responses in the groups from 24–32 h (Mann-Whitney U test).

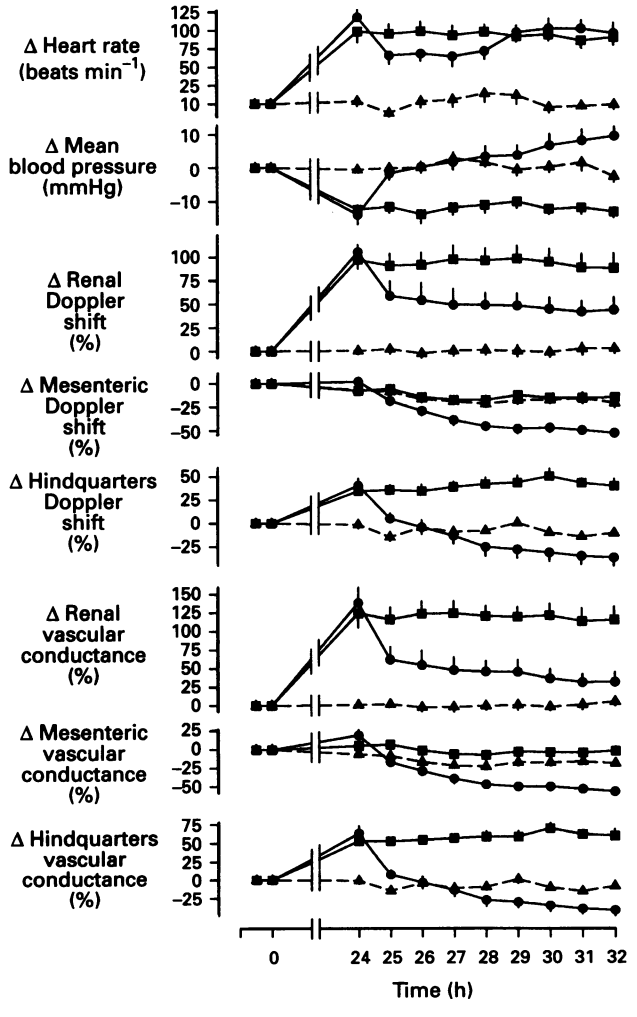

Figure 7 Regional haemodynamic changes in conscious Long Evans rats receiving a saline infusion for 32 h (▲, $n=8$, Group A), or LPS (150 μg kg^{-1} h^{-1}) for 32 h together with saline for the last 8 h (■, $n=8$, Group C), or LPS for 32 h together with N^G-nitro-L-arginine methyl ester (L-NAME (3 mg kg^{-1} h^{-1}) for the last 8 h (●, $n=8$, Group M). Values are mean with s.e.mean; statistics are given in the text for clarity.

There was a reduction in the tachycardia (1–4 h), but not below baseline. The LPS-induced increases in renal flow and vascular conductance were suppressed (1–8 h), but substantial hyperaemia and vasodilatation persisted (Figure 7). In contrast, the increases in hindquarters flow and vascular conductance were abolished (both 1–3 h), and thereafter these variables were reduced below baseline (both 4–8 h) (Figure 7). As before, LPS caused no change in mesenteric flow, and only a slight mesenteric vasodilatation at 24 h; L-NAME caused substantial reduction of mesenteric flow and vascular conductance below baseline (both 1–8 h) (Figure 7).

Cardiac haemodynamics (Group C) Resting cardiovascular variables are shown in Table 2.

L-NAME reversed the LPS-induced hypotension (1–3 h), and thereafter caused a rise in MAP (4–8 h) (Figure 8); although the tachycardia was reduced (3–8 h), a substantial increase in heart rate remained (Figure 8). The elevation in cardiac index was reduced by L-NAME (1–8 h), and cardiac index was not different from baseline from 4–8 h (Figure 8). L-NAME abolished the rise in stroke index (1–6 h), and subsequently reduced it below baseline (7 and 8 h) (Figure 8). The LPS-induced increase in peak aortic flow was diminished (1–7 h), and by the end of the observation period peak aortic flow was below baseline (Figure 8). A similar pattern of change was seen for dF/dt_{max}, although L-NAME did not reduce this variable below baseline (Figure 8). L-NAME reduced the LPS-induced rise in total peripheral conductance (1–5 h) and thereafter caused vasoconstriction (6–8 h) (Figure 8). Although L-NAME caused a reduction in central venous pressure (1–8 h) this was not below baseline (Figure 8).

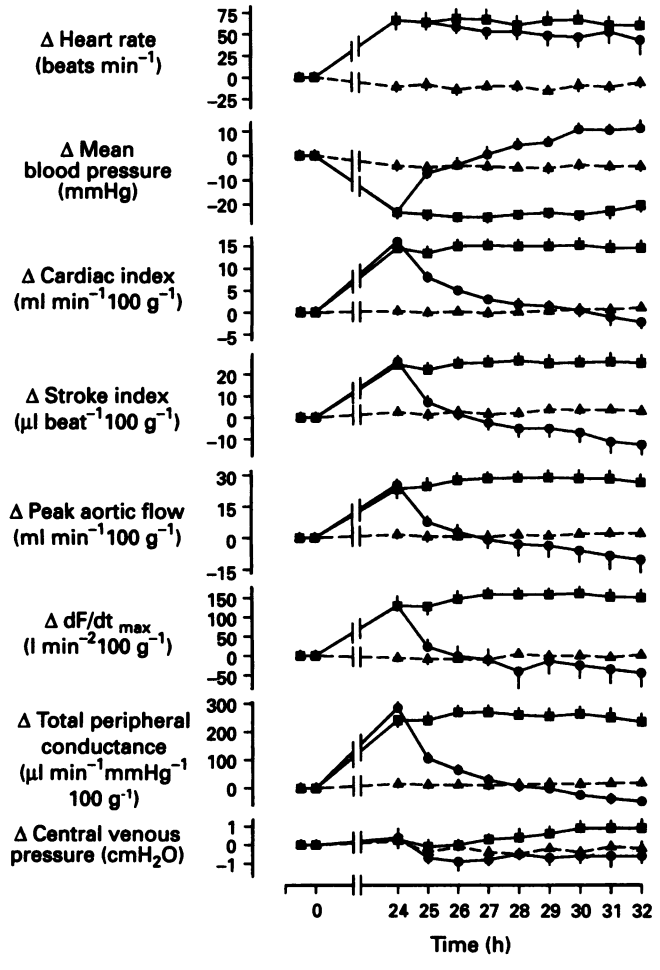

Figure 8 Cardiac haemodynamic changes in conscious Long Evans rats receiving saline infusion (▲, $n=8$, Group A) for 32 h or LPS (150 μg kg^{-1} h^{-1}) infusion for 32 h together with saline for the last 8 h (■, $n=8$, Group B), or LPS for 32 h together with N^G-nitro-L-arginine methyl ester (L-NAME, 3 mg kg^{-1} h^{-1}) for the last 8 h (●, $n=8$, Group C). Values are mean with s.e.mean; statistics are given in the text for clarity.

Discussion

We have recently described the profile of regional haemodynamic changes during a continuous 24 h infusion of LPS (150 μg kg^{-1} h^{-1}) in conscious rats (Waller *et al.*, 1994a). The present work extends that published study by showing the regional haemodynamic changes present at 24 h are stable during a subsequent 8 h period of LPS infusion, and, additionally, describes the accompanying changes in cardiac haemodynamics. Thus, during the early (0–3 h) phase of LPS infusion, there was slight hypotension and tachycardia, accompanied by reductions in cardiac and stroke index, but marked, and selective, increases in renal flow and conductance. Our finding of an early, marked renal hyperaemia in response to LPS is at variance with other studies (e.g., Kikeri *et al.*, 1986; Fish *et al.*, 1986; Churchill *et al.*, 1987; Burnier *et al.*, 1988; Weber *et al.*, 1992), but the accompanying reduction in cardiac index is consistent with the effects of LPS in conscious sheep (Noshima *et al.*, 1993).

During the middle phase of LPS infusion (3–8 h), there was still only a very modest hypotension, but a progressive tachycardia, accompanied by increases in cardiac and stroke index, peak aortic flow, dF/dt_{max} and total peripheral conductance. Central venous pressure was reduced, consistent with venodilatation (Vallance *et al.*, 1992), and the marked renal hyperaemic vasodilatation was maintained. This picture is of a hyperdynamic circulation, as described recently in LPS-treated conscious sheep (Traber *et al.*, 1988; Sugi *et al.*, 1991; Meyer *et al.*, 1992; Weber *et al.*, 1992; Noshima *et al.*, 1993). In those studies, estimates of cardiac function indicated that, in spite of an elevation in cardiac output, there was myocardial depression. In the present work we observed an increase in dF/dt_{max}, but since the latter may be influenced by afterload (de Wildt & Sangster, 1983), it is feasible that the increase in cardiac index we observed was due to the peripheral vasodilatation. It is notable that, even at this stage, the latter was seen in the renal, but not mesenteric or hindquarters, vascular beds. These findings are, superficially, consistent with the observation that LPS does not cause hyporesponsiveness to vasoconstrictors in the mesenteric vascular bed *ex vivo* (Mitchell *et al.*, 1993), although this is not the case *in vivo* (Waller *et al.*, 1994a).

Between 8 and 24 h, the responses to LPS became maximal, with clear hypotension, tachycardia and increases in all measured haemodynamic variables, with the exception of mesenteric flow and vascular conductance, and central venous pressure. Once again, this is similar to the hyperdynamic circulation described in LPS-treated conscious sheep (see earlier), but in those studies no measurements were made of regional haemodynamics. This is an important point because it is clear that, in the conscious rat model described here, there were substantial differences in the regional haemodynamic responses to LPS.

Although the lower dose of LPS (15 μg kg^{-1} h^{-1}) still caused substantial increases in renal flow and vascular conductance, it did not cause the widespread and progressive hyperdynamic sequelae described above, when LPS was infused at 150 μg kg^{-1} h^{-1}, so the latter regime was that used in all subsequent studies. Measurement of tissue iNOS activity and plasma NO_x showed clear increases between 2–6 h after the onset of LPS infusion, but by 24 h, when haemodynamic changes were greatest, tissue iNOS activity and plasma NO_x were falling. Moreover, it is notable that, although the increases in renal flow and vascular conductance were the most striking feature of the haemodynamic changes seen during LPS infusion, there were no significant changes in iNOS activity in the kidney. Of

course, it is feasible that biochemical measurement of gross tissue iNOS activity fails to detect changes of iNOS activity in the microcirculation, and hence is a less sensitive indicator of the functional involvement of NO than is the assessment of haemodynamic changes. Hence, it could be argued that a better way of quantitating the contribution of NO to the cardiovascular sequelae of endotoxaemia would be by measuring responses to NOS inhibition; we did this by utilising L-NMMA and L-NAME.

In the first series of experiments, we pretreated animals with different doses of L-NMMA. Clearly, pretreatment is not a strategy that could usually be employed clinically, but from the point of view of determining factors responsible for LPS-induced changes, it seemed rational. The higher doses of L-NMMA (10 and 30 mg kg^{-1} h^{-1}) abolished the early (2 h) renal vasodilatation seen during LPS infusion, although there was an enhanced fall in arterial blood pressure, possibly due to a reduction in cardiac output. These findings could indicate an involvement of NO (via the constitutive enzyme) in some of the early haemodynamic changes (Thiemermann & Vane, 1990; Szabo *et al.*, 1993), but, clearly, these doses of L-NMMA did not produce selective suppression of hypotensive and vasodilator responses to LPS. Indeed, the vasoconstrictions in the mesenteric and hindquarters vascular beds in the combined presence of L-NMMA and LPS were greater than occurred with L-NMMA alone. While this could have been due to NOS inhibition simply unmasking the vasoconstrictor actions of sympathoadrenal activity, angiotensin II, vasopressin (Schaller *et al.*, 1985) and endothelin (Sugiura *et al.*, 1989), another possibility should be considered. There is evidence that, in conscious normal rats, a component of the pressor and regional vasoconstrictor effect of L-NMMA is inhibited by the endothelin antagonist, bosentan (Gardiner *et al.*, 1995). Hence, it may be that, with L-NMMA pretreatment, the LPS-induced release of endothelin (Sugiura *et al.*, 1989) is augmented.

The only dose of L-NMMA given as a pretreatment (3 mg kg^{-1} h^{-1}) which did not cause serious reductions in regional blood flows had no marked effect on any of the LPS-induced changes, presumably because the dose was too low. Since, during LPS infusion in the presence of the higher doses of L-NMMA there were such marked reductions in regional flows, and the animals showed such signs of malaise, the protocols were run for only 8 h; hence these experiments did not allow us to assess any putative involvement of NO during the later stages of LPS infusion. However, our findings indicate that during the first 8 h of LPS infusion any effects of NO might be beneficial, rather than detrimental.

Intervention with L-NMMA after 4 h of LPS infusion (i.e., at a time when iNOS activity was increasing) clearly affected the haemodynamic status of the animals after 24 h of LPS infusion since there was no hypotension, no hindquarters vasodilatation, and a greatly attenuated renal vasodilatation. This could indicate that the induction of iNOS (measured at 6 h) was responsible for the haemodynamic events occurring between 6 and 24 h. However, the fact that iNOS activity had decreased when the vasodilatation was maximal (at 24 h) suggests that NO was not directly responsible for the vasodilated state at that time – rather that another process, possibly triggered by NO produced through iNOS activity, was involved.

The latter suggestion is consistent with the results obtained when L-NMMA was given after 24 h of LPS infusion. Thus, if NO had been directly responsible for the vasodilatation, then administration of L-NMMA should have caused a straightforward reversal of the condition; indeed, enhanced responsiveness to L-NMMA may have been anticipated (Klabunde & Ritger, 1991; Klabunde & Helgren, 1992). On the contrary, the LPS-infused animals were less sensitive to L-NMMA than normal animals. There are several possible explanations for this. It is becoming increasingly clear that the vasoconstrictor effect of NOS inhibitors may not only be due to inhibition of basal NO production, but may also be due to disinhibition of endothelin release (see above). It is feasible, therefore, that the reduced sensitivity of the LPS-infused animals to L-NMMA was due to a downregulation of the endothelin system at this stage. Alternatively, since L-NMMA is converted to L-arginine (Hecker *et al.*, 1990), then an upregulation of this conversion process could have resulted in enhanced breakdown of L-NMMA, further production of NO, and thus a diminished sensitivity to L-NMMA. It was notable, however, that the renal vascular bed of the LPS-infused animals actually showed a greater vasoconstrictor response to L-NMMA, possibly due to activation of mechanisms such as the renin-angiotensin system (see above).

Using the non-selective NOS inhibitor, L-NAME, it was possible to reverse most of the haemodynamic effects of LPS, but not the tachycardia. If L-NAME were exerting anti-muscarinic effects (Buxton *et al.*, 1993) this could interfere with the cholinergic vagal control of heart rate. However, in conscious rats a dose of L-NAME (1 mg kg^{-1} h^{-1}) sufficient to raise mean arterial blood pressure by 23 ± 3 mmHg had no effect on cardiac baroreflex sensitivity (Gardiner *et al.* 1991).

Meyer *et al.* (1992) using a similar model to ours (i.e., continuous low-dose LPS infusion), but in sheep, have shown reversal of the hyperdynamic state with L-NAME, but they indicated the need to examine regional perfusion. This we have done, and here we show that the reversal of the modest hypotension was at the expense of seriously reduced mesenteric blood flow and reduced stroke index. It is likely that this was due to L-NAME inhibiting both constitutive and iNOS, since we have found recently that pretreatment with aminoguanidine, a more selective inhibitor of iNOS, suppressed the renal, mesenteric and hindquarters vasodilatation seen after infusion of LPS for 24 h, but did not precipitate overt vasoconstriction at that stage (Waller *et al.*, 1994b), or influence hyporesponsiveness to acetylcholine or methoxamine (Waller *et al.*, 1995). Collectively, our results indicate that factors in addition to iNOS must contribute to the vasodilatation seen after LPS infusion for 24 h.

In summary, we have shown it is possible to reproduce all the cardiovascular signs of hyperdynamic endotoxaemia by continuous infusion of LPS in conscious rats. It is notable that, in the continued presence of LPS, increased tissue iNOS activity and plasma NO_x levels are not sustained, and hence there is a dissociation between these changes and the haemodynamic sequelae. Moreover, a variety of treatment regimes with L-NMMA or L-NAME do not simply reverse the cardiovascular effects of LPS; indeed, in this model, it appears that such NOS inhibitors may be detrimental. However, it is feasible that in clinical endotoxaemia, patients would experience intermittent exposures to LPS, and hence show profiles of iNOS activity different from that seen here. Moreover, although it seems unlikely from our findings that non-selective NOS inhibition would be beneficial during the hyperdynamic circulatory phase of endotoxaemia, we cannot discount the possibility that such interventions with selective iNOS inhibitors would be therapeutic in endotoxaemic patients who had passed into the subsequent, hypodynamic circulatory state.

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